

## EVALUATION OF ANTI-INFLAMMATORY EFFECT OF IBUPROFEN USING WITEPOSOL H15 RECTAL SUPPOSITORIES VERSUS ORAL ROUTE

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### ABSTRACT

Inflammation is the self-protective reaction of tissues towards infection, irritants, or foreign substances. Though it is a part of host defense mechanism, when it becomes severe, it turns out to be a hopeless condition which causes damage of tissues; hence control of inflammation becomes essential. This paper presents a study of anti-inflammatory effect of ibuprofen-witepsol H15 rectal suppositories versus oral route of administration using intra-peritoneal (IP) route as positive control. Suppositories were prepared using fusion method and tested for physico-chemical properties according to British Pharmacopeia (B.P) 2007 procedures. Screening for anti-inflammatory activity of ibuprofen was carried out against carrageenan-induced rat paw oedema in wistar strain albino rats ( $200 \pm 20$  g) of either sex. The formulated suppositories were found to satisfy B.P, requirements for weight variation, liquification time, disintegration time, hardness values and content uniformity. The entrapment efficiency (%) of ibuprofen was 99-100 % w/w for all the tested suppositories. The increase in paw oedema volume significantly ( $p < 0.0001$ ) inhibited using ibuprofen-witepsol H15 rectal suppositories compared to control. The total area under the curve (AUC) for paw volumes time curve were reduced from 5.54 ml/h for control to 3.45, 4.38 and 4.78 ml/h using rectal suppositories, oral and IP routes. The anti-inflammatory inhibition (%) achieved after 4-5 hours was 40.58, 29.87 and 65.87, after rectal suppositories, oral and I.P routes of administration respectively. The total AUC (%) of inhibition were 33.81, 27.07 and 62.16 at 4 and 5 hours. using, rectal suppositories, oral and IP routes of administration respectively. The results justified the use of suppositories in the treatment of inflammation when oral route is unreliable.

**KEY WORDS:** Ibuprofen, Anti-inflammatory activity, Suppository base, Oral route, Paw oedema

### INTRODUCTION

Inflammation is the self-protective reaction of tissues towards infection, irritants, or foreign substances<sup>(1)</sup>. It is a part of host defense mechanism, when it becomes severe, it turns out to be a hopeless condition which causes damage of tissues. Chronic inflammation is associated with certain severe disease like rheumatoid arthritis, type II diabetes, Alzheimer's disease<sup>(2)</sup>. Non-steroidal anti-inflammatory drugs (NSAIDs) are the available potent synthetic drugs in the treatment of inflammatory diseases but<sup>(3)</sup>. The prolonged oral uses of NSAIDs are well known to be associated with peptic ulcer formation. Rectal route using suppositories can be a promising alternative approach for administration of these drugs. Rectal route provides reduction of side effects namely gastrointestinal irritation and the avoidance of both disagreeable taste and first pass effect<sup>(4-7)</sup>. Rectal administration can also be an alternative route when oral route is not possible in nausea, vomiting, and unconscious conditions<sup>(8,9)</sup>. Comparison of the oral administration of ibuprofen with rectal suppositories revealed that ibuprofen suppositories can be considered to management of fever and pain when the oral route is not available<sup>(10)</sup>. In addition, NSAIDs are usually good candidates for the development of conventional or controlled release preparations particularly through the rectal route. Carrageenan rat paw oedema model

is traditionally used for search and development of new NSAIDs with assessment of effects after oedema induction<sup>(11,12)</sup> neglecting long-term effects<sup>(13,14)</sup>. Therefore, the aim in this study is to assess the anti-inflammatory activity of ibuprofen recta suppositories in comparison to oral route using intra-peritoneal routes as positive control.

### MATERIAL AND METHODS

Ibuprofen was from Prodotti Chimici Industrial, Italia. Witepsol H15, (WH15) was from Dynamit Nobel, Witen, Germany. Carrageenan sodium was from (BDH, England). Water for injection (2 ml) ampoules was bought from local pharmacy. All other chemicals used in this work were from Sigma-Aldrich Co. (St. Louis, MO, USA) and used as received.

#### Animals:

Wistar strain albino rats weighing  $200 \pm 20$  g of either sex were used in the study. The animals were maintained on standard diet and tap water. The temperature and humidity were kept at optimum and the animals were exposed to natural day-night cycles. The study was conducted in accordance with the nationally accepted guidelines for laboratory animal use and care which was approved by Animal Ethical Committee (NMRC35/2009).

#### Preparation of Suppositories:

Suppositories plain and containing 15 mg/kg of ibuprofen were prepared using the fusion method.<sup>(15)</sup> Briefly, Witepsol H15 base in absence and presence of ibuprofen were melted and poured into six cavities metal mould. The prepared suppositories were left for 24 hrs. at 25°C before testing. Displacement value for

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ibuprofen was calculated based on the following equation:

$$f = (100 \times (E - G)) / (G \times x) + 1.$$

Where f is the displacement value, E is the weight of the suppository without ibuprofen, G is the weight of the suppository with active substances and x is the active substance content in percentage<sup>(16)</sup>.

#### Characterization of Suppositories:

##### Weight Uniformity:

Weight uniformity test was carried out according to British Pharmacopoeia B.P 1998 method<sup>(17)</sup> Briefly, 20 suppositories were individually weight and the average weight then calculated. There must be not more than two suppositories differ from the average weight by more than 5% and no suppository differs from the average weight by more than 10%.

##### Liquefaction Time:

The ascending melting point method was used to determine the melting point of each suppository<sup>(18)</sup>. Briefly, capillary tubes of 10 cm in length sealed at one end were filled with the formulation to about 1cm height. The tubes then dipped in gradually heated electro-thermal thermometer from which the temperature for melting of suppositories was predicted.

##### Disintegration Criteria:

Disintegration test was performed on six suppositories according to British Pharmacopoeia 2007<sup>(19)</sup> using USP tablet disintegration (Model PTW, Germany) test apparatus. Disintegration time (D.T.) for suppositories was determined in water maintained at  $37 \pm 0.5^\circ\text{C}$ .

##### Hardness Test:

Hardness test was performed using Erweka hardness tester. The temperature inside the testing chamber was maintained at  $25^\circ\text{C}$  by means of circulating water from thermostat connected to the tester. The suppository was placed into the holding device with the tip upwards and the test chamber was then closed with glass plate. At this point, the initial load, which was given by the entire suspended block, was 600 gm. Subsequently every minute a disk of 200 gm. was added until the suppository crush under the load of the weight. The mass required to crush the suppository was calculated by the summing the masses weighing on the suppository when it was collapsed (including the initial mass of the device i.e. 600 gm.)<sup>(20)</sup>.

##### Content Uniformity:

Ibuprofen content determination was done using phosphate buffer pH 7.4 as solvent medium. Three randomly selected suppositories from each formulation were taken in 1000 ml flask containing 100 ml phosphate buffer pH 7.4. The flask was shaken until the suppositories completely dissolved. Samples of the resulting solutions were appropriately diluted, filtered through doubled layer Whatman filter paper followed by  $0.45 \mu\text{m}$  disc filter and subjected to absorbance measurement on Shimadzu PR240, Kyoto, Japan UV/Vis spectrophotometer at 264 nm using suppository solution prepared without ibuprofen as a blank. Ibuprofen content was calculated using calibration curve equation obtained by plotting the ab-

sorbance for serial concentrations of ibuprofen in the phosphate buffer pH 7.4<sup>(21)</sup>.

#### Anti-Inflammatory Studies:

The method of Winter *et al.*<sup>(22)</sup> was adopted to screen the anti-inflammatory activity of ibuprofen against carrageenan-induced paw oedema in Wistar strain Albino rats. Prior to start of the experiment, the body weight of animals was recorded individually for evaluating proper treatment dosage and animals, divided into four groups of six each. Group I received distilled water and served as -ve control. Group II received the test drug, ibuprofen by oral route at dose of 2 mg/ml in water. Group III: rats received intraperitoneal (I.P) injection of ibuprofen (2mg/ml) in water for injection and served as +ve control. Animals in Group IV, received treatments with rectal WH15-ibuprofen suppositories respectively. After one hour, 50  $\mu\text{l}$  of freshly prepared 1%  $\lambda$ -carrageenan sodium in saline was injected into the sub-plantar region of the right hind limb of the tested rats. Paw volume was measured by volume displacement method using a digital plethysmometer (Ugo Basile, Italy) immediately and after 0.5, 1.0, 2.0, 3.0, 4.0, 0.5, 6.0 and 8.0 hour of  $\lambda$ -carrageenan sodium injection. A significant reduction in the paw volume compared to untreated control animals was considered as the in vivo anti-inflammatory response.

#### Calculation:

The change in paw oedema volume for each time interval was calculated using the following equation:

$$\text{Volume of oedema} = V_f - V_i$$

where  $V_f$  and  $V_i$  are the volumes of the paw oedema after and before Carrageenan sodium injection. The percentage (%) of inhibition of inflammation for each time interval was calculated applying the following equation:

$$\% \text{ inhibition of inflammation} = \text{NC-T} / \text{NC} \times 100$$

where NC refers to negative control and T refers to the test groups.

The area under the curve for oedema volume curve of the time-course was calculated using the trapezoidal rule.

$$AUC = \sum_{i=1}^n \frac{(V_{i-1} + V_i)}{2} (t_i - t_{i-1})$$

where  $V_i$  is paw oedema volume at  $t_i$

The level of inhibition response of inflammatory was calculated using AUC according to the following relation:

$$\% \text{ Inhibition. of. oedema} = \left( \frac{AUC_{\text{control}} - AUC_{\text{test}}}{AUC_{\text{control}}} \right) \times 100$$

#### Statistical Analysis:

Results of anti-inflammatory activity were expressed as Mean increase in paw diameter  $\pm$  SEM (standard error of the mean). Results were analyzed using one-way analysis of variance (ANOVA) followed by t test using Graph Pad software 2016 Quick Calcs. Differences were considered as statistically significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

In this work, ibuprofen-suppositories were formulated using Witepsol H15 as fatty base. The formulated rectal suppositories were studied for anti-inflammatory activity of ibuprofen in comparison to

oral administration using IP route as positive (+ve) control. (Table 1) shows the preliminary physico-chemical evaluation results of suppositories using British Pharmacopoeia (BP) 2007 procedures.

(Table 1) Physicochemical Characterisation data of Ibuprofen Witepsol H15 Suppositories.

Formula	Weight mg± SD n=20	Liquefaction Time (min)	Hardness (Kg)	Disintegration Time (min)	Content Uniformity (mg) ± SD	Entrapment efficiency (%w/w)
WH15	1.70 ± 0.20	9.0	2.4	11	0.00	0.00
WH15+Ibuprofen	1.69 ± 0.05	8.5	2.0	9	300.2 ± 0.76	100.00

WH15=Witepsol H15,

The weight of suppositories for different formulations was found to comply with British Pharmacopoeia (BP) standards<sup>(19)</sup>. The percentage deviation in weight of all prepared suppositories was less than 0.5 from the average weight. Liquefaction time was ≤ 9.0 min for the formulated suppositories. This value is within the acceptable limit (30 minutes) required for complete melting<sup>(17)</sup>. Hardness values for the tested suppositories were ≤ 3 kg/cm<sup>2</sup> indicating good mechanical strength with an ability to withstand physical and mechanical stress condition while handling. Disintegration time was 11 min for plain suppositories reduced to 9.0 min after incorporation of ibuprofen. The content of ibuprofen in different suppository formulations was highly uniform with the entrapment efficiency (%) was 99-100 % w/w that is within the limits (95-105%) specified by British Pharmacopoeia, BP<sup>(17)</sup>. Carrageenan induced paw oedema is a well-

established model to test effect of drugs against acute inflammatory response<sup>(23)</sup>. The inflammatory response generated in this model is considered to be biphasic in nature with a preliminary oedema generation caused primarily by the effect of hypersensitivity mediators like histamine, serotonin and bradykinin on vascular endothelium, which is followed by a delayed response by the release of prostaglandins and nitric oxide<sup>(24)</sup>. The model have been used by several investigators to search for new anti-inflammatory product<sup>(25,26)</sup>. In the present work the model was applied to evaluate the anti-inflammatory activity of ibuprofen-rectal suppositories in comparison to oral ibuprofen using IP route as a positive control. (Table 2) illustrates the change in the paw oedema volume for each group of animals and the percentage inhibition with the treatment compared to control.

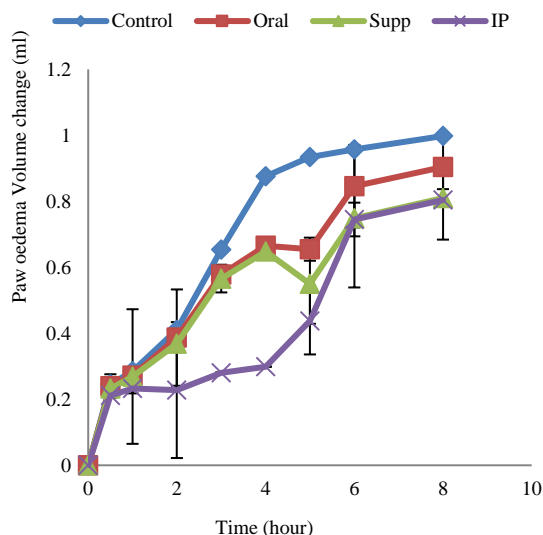
(Table 2) Paw oedema volume changes and Anti-inflammatory efficiency of deferent ibuprofen formulations.

Treatment	Change in paw oedema volume (ml) and inhibition (%) at respective time (hour) ± SEM							
	0.5	1	2	3	4	5	6	8
-ve control	0.245 ± 0.076	0.285 ± 0.017	0.410 ± 0.005	0.654 ± 0.0046	.876 ± 0.006	0.934 ± 0.010	0.957 ± 0.027	0.998 ± 0.038
Oral Route	0.240 ± 0.036 (2.04)	0.272 ± 0.026 (4.56)	0.387 ± 0.146 (5.61)	0.578 ± 0.023*** (11.62)	0.665 ± 0.009*** (24.1)	0.655 ± 0.035*** (29.87)	0.846 ± 0.125 (11.60)	0.904 ± 0.102 (9.42)
IP (+ve control)	0.211 ± 0.006*** (13.9)	0.233 ± 0.015** (18.24)	0.228 ± 0.206 (44.4)	0.280 ± 0.002*** (57.18)	0.299 ± 0.001*** (65.87)	0.437 ± 0.101*** (53.21)	0.745 ± 0.051*** (22.15)	0.804 ± 0.12** (19.44)
WH15 supp.	0.233 ± 0.021 (4.9)	0.269 ± 0.204 (5.6)	0.369 ± 0.001*** (10)	0.566 ± 0.042** (13.46)	0.648 ± 0.004*** (26.02)	0.550 ± 0.121*** (40.58)	0.749 ± 0.21* (21.73)	0.810 ± 0.027*** (18.84)

Values are expressed as the mean and ± SEM (n=6) while values in parenthesis represent the percentage inhibition of paw oedema \*p<0.05, \*\*p<0.01, \*\*\*p < 0.0001 compared with vehicle control (T test) WH15, Witepsol H15. -ve control (without Ibuprofen). +ve control (Intra-peritoneal, IP) rout.

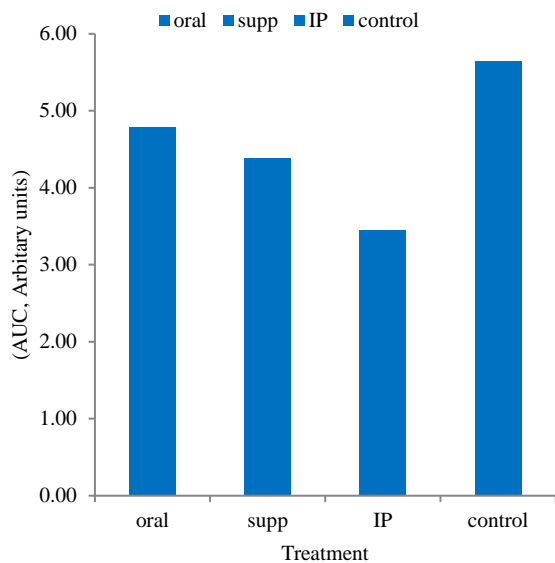
All the treated ibuprofen formulations showed significant (<0.05) anti-inflammatory activity reflected in the significant reduction of the oedema volume compared to control. The paw volume was significantly reduced (p<0.0001) after 0.5, 2 and 3 hours following IP injection, rectal suppositories and oral administration of ibuprofen. The change in paw oedema volume measured at 8 hours of administration of ibuprofen was 0.998 ± 0.038 ml (control) reduced to 0.810 ± 0.027 ml (rectal suppositories), 0.904 ± 0.102 ml (oral) and 0.804 ± 0.12 ml (IP). The results showed that before 2 hours post-ibuprofen suppository treatment, unlike IP route which produced significant reduction in oedema; there was no significant difference between the paw oedema in the ibuprofen-rectal

suppository, oral treated rats and control. However, from 2 up to 8 hours post-treatment, there were reductions in paw oedema, reaching statistical significance (p < 0.05) using rectal suppository compared to control. In contrast, ibuprofen given by oral route showed significant reduction in oedema at 3, 4 and 5 h post treatment but become insignificant at 6 and 8 hours of administration. IP ibuprofen pretreatment, however, produced more significant (Student's t-test) reduction in oedema formation in rats compared to the rectal suppository. Ibuprofen suppositories showed stronger inhibition of oedema, as compared to oral route and closely to IP route at the end of six hours (figure 1).



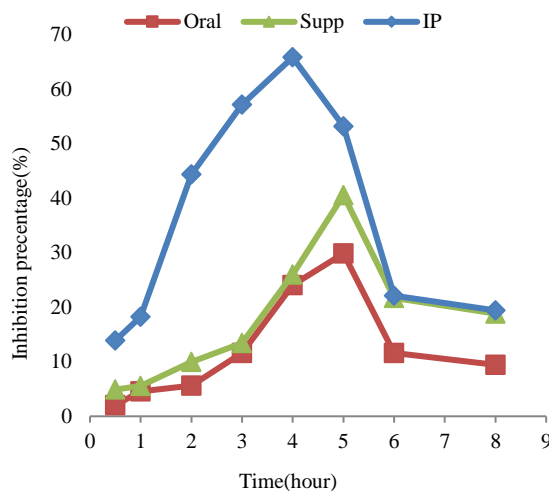
**(Figure 1)** Paw oedema volume changes after oral, rectal suppository (Supp.) and Intra-peritoneal (IP) administration of ibuprofen. Values are expressed as mean ± SEM, n = 6, \*p < 0.05 compared to control with all the groups.

Based on the total AUC for the paw volume changes time curve for the tested formulations, the routes of administration can be ranked in the descending order of IP > rectal > Oral (figure 2).



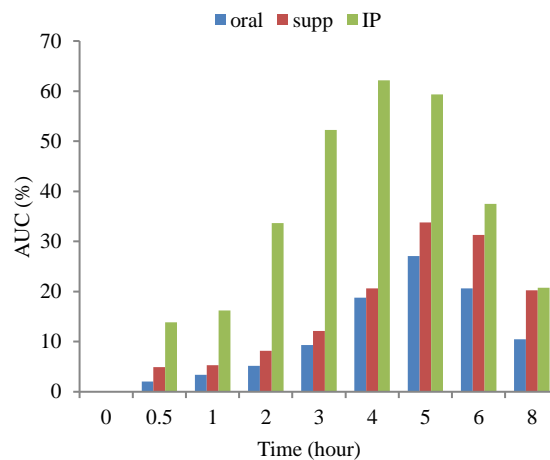
**(Figure 2)** The calculated AUC for volume change time curve up to 8 h after induction with carrageenan following oral, rectal suppository (Supp.) and Intra-peritoneal (IP) administration of ibuprofen.

The AUC was reduced from 5.54 ml/hour for control to 3.45, 4.38 and 4.78 ml/hour after IP, rectal and oral route of administration. The maximum percentage of inhibition calculated based on the paw volume changes data was 65.87 (%) at 4 hours of treatment using IP, 40.58 (%), at 5 hours using rectal suppositories and at 29.87 (%) using oral route (figure 3).



**(Figure 3)** The anti-inflammatory inhibition (%) of ibuprofen after oral, rectal suppositories (Supp) and intra-peritoneal (IP) administration.

The high inhibition (%) for IP administration of ibuprofen can be explained by the availability of the anti-inflammatory drug after IP administration route compared to rectal and oral routes. The delayed absorption of ibuprofen lead to that maximum oedema volume reduction almost occurs after four hours of Carrageenan injection. Ibuprofen suppositories exhibited more prominent inhibition of oedema in the later stages of inflammation i.e. after four hours of treatment compared to oral ibuprofen. Despite the fact that the use of suppository statistically showed no significant differences ( $p > 0.05$ ) from oral route for administration of ibuprofen in reducing the paw edema volume up to 4 hours of administration, rectal suppositories showed significantly ( $< 0.0001$ ) inhibition effect even similar to IP route after 4 hours. of administration. Similarly, based on the total AUC measured for the three routes for administration of ibuprofen, the percentage inhibition (%) can be ranked order of IP > rectal > Oral (figure 4).



**(Figure 4)** Area under curve (AUC) inhibition (%) of ibuprofen after oral, rectal suppository (Supp.) and intra-peritoneal (IP) administration.

### CONCLUSION

Inflammation is the self-protective reaction of tissues towards infection, irritants, or foreign substances. Oral administration of anti-inflammatory NSAIDs such as ibuprofen usually associated with side effects including irritation of gastrointestinal tract. Though search for alternative route of administration is justified. Rectal administration of ibuprofen-WH15 rectal suppositories to wistar strain albino rats showed significant inhibition of oedema compared to control and better than oral route. The results justified the use of suppositories in the treatment of inflammation when oral route is unreliable. Carrageenan induced paw oedema method proved to be suitable for predicting the anti-inflammatory of ibuprofen using different routes of administration.

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# MDCTA OF LOWER EXTREMITIES ARTERIES STENO-OCCLUSION IN VARIOUS AGE GROUPS AND BOTH GENDERS: STUDY INCLUDED 38 PATIENTS PRESENTED WITH CLINICAL MANIFESTATIONS

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## ABSTRACT

The purpose of our study was to evaluate the role of Multi Detector Row Computed Tomography Angiography (MDCTA) in evaluating the Peripheral Arterial Disease (PAD) affecting the lower limb for Libyan patients presented with clinical manifestations. Thirty-eight patients referred in the period from December 2017 to March 2018 for Computed Tomography Angiography (CTA) of lower limb vessels to evaluate and diagnose the diseases of peripheral lower extremities arteries. For evaluation of the lower extremities arterial diseases 128-Row MDCT (Toshiba Aquilion, Japan) was used. After preparation of the patients, we used intravenous line for non-ionic iodinated contrast injection and the patients were scanned from renal level to the toes who took 20-30 seconds for scanning. Three-dimensional (3-D) visualization was essential. Thirty-eight patients with clinical manifestations referred for CTA of lower limbs arteries were with atherosclerotic steno-occlusive disease. 28 patients had claudication, 34 patients had rest pain with ischemia, 38 patients had Diabetes Mellitus (DM) and only two patients were with trauma. The scanning of the lower extremities' arteries showed singular or multiple atherosclerotic stenosis or occlusion. The largest group among the referred patients with clinical manifestations was diabetic mellitus group and the smallest one was trauma group. MDCTA is preferred tool for evaluation of lower extremities arteries of patients with clinical manifestation. The arterial steno-occlusion increased by increasing age and more among males than females. Highly detected lower extremities' arteries of atherosclerotic steno-occlusive disease were in patients with clinical manifestations (claudication, rest pain with ischemia, diabetes mellitus and trauma).

**KEY WORDS:** MDCTA, Patients, Age, Gender, Clinical manifestations, Lower extremities arteries, Steno-occlusion.

## INTRODUCTION

The common femoral artery is the arterial supply of the lower limb arising from the external iliac artery. It begins at inguinal ligament level and gives profunda femoris and continues as superficial femoral artery. Its continuation is the popliteal artery at level of adductor canal which dividing into anterior tibial artery and posterior tibioperoneal trunk. The anterior tibial artery passes to reach the anterior leg and continues to the foot dorsum as dorsalis pedis artery. The tibioperoneal artery is divided into posterior tibial artery and peroneal artery. The posterior tibial artery divided into medial and lateral planter arteries<sup>(1)</sup>.

Aortic and lower limb arterial disease is a common disease and refers to disease of blood vessels supplying the extremities reflecting increasing prevalence of atherosclerosis. The (PAD) leading to obstruction of the peripheral artery may associated with symptoms and signs indicating ischemia. Most of patients with symptoms of claudication have lower extremity disease. The atherosclerotic obstructions of distal aorta and lower extremity arteries are the most common conditions of peripheral arterial obstructive diseases<sup>(2,3,4,5)</sup>.

Digital Subtraction Angiography (DSA) is an invasive, cost and has some morbidity and it provides a 2-dimensions (2-D) map of the vessels which may be not accurate for evaluate the degree of vascular stenosis and tortuosity. DSA is more used for therapeutic interventions rather than diagnostic studies<sup>(2,6)</sup>.

CTA and Magnetic Resonance Angiography (MRA) provide a high resolution, 3-dimensions (3-D) and it

is a robust, non-invasive procedure for evaluation and diagnosis of peripheral arterial disease as well as decrease morbidity and imaging cost<sup>(6,7,8)</sup>. Radiation exposure of DSA is 3.9 times greater than with CT angiography<sup>(8)</sup>.

MRA generally used for young patients and for patients with contrast allergy or renal insufficiency. MRA does not acceptable for assessment of PAD for patients with pacemaker or other implants, as well as it is not effective for uncooperative patients<sup>(9)</sup>.

Developments of multi-detector computed tomography angiography MDCTA made accurate assessment of the peripheral arteries and increased use to depict the peripheral vessels runoff. Multi-Planer Reformations (MPRs) were required in patients with extensive calcification providing accurate steno-occlusive assessment<sup>(10,11,12)</sup>.

Calcification is widely involving the arterial walls and patients with a history of diabetes mellitus, cardiac disease or elderly patients are likely to have extensive calcification<sup>(13)</sup>.

MDCTA is superior to DSA visualization of arterial territories. It has major advantages compared to DSA in the detection of eccentric lesions (Figures 1,2) of PAD with use of cross sectional multi-planer reformatted CTA<sup>(14,15,16)</sup>.



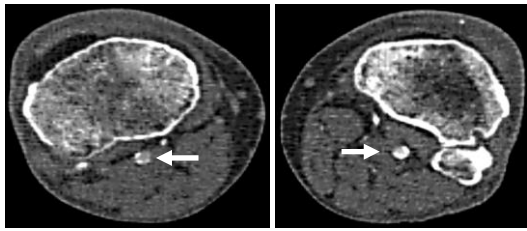
(Figure 1) 80 yrs patient with eccentric lesion at the right iliac artery (white arrow).

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(Figure 2) 80yrs patient with eccentric lesions at both posterior tibial arteries (white arrows).

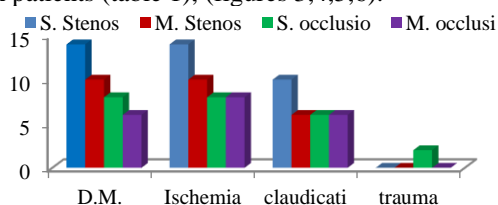
**MATERIALS AND METHODS**

Between December 2017 and March 2018, the clinicians in Zliten Teaching Hospital referred 38 patients (31 men, 7 women; mean age 61 years; age range 30-81 years) with suspicion of lower limb vascular disease for CTA of the lower extremities arteries inflow and runoff with complaint of claudication, rest pain, trauma, soft tissue ulceration and ischemia. Underwent multi-detector computed tomography angiography scanner (128 row MDCTA Toshiba Aquilion, Tokyo-Japan) for the referred patients. Pump injector of automated contrast monitoring power was the accessory tool to complete examinations. After patients' preparation performed injection of 140 ml contrast material via inserted canula at a rate of 4.5 ml/sec.

**RESULTS**

The patients referred to our department with clinical manifestations for doppler ultrasound study of the lower limbs arteries. The studied cases with clinical manifestations were 38 patients (mean age 61 years; 81% males and 19% females). Regarding to clinical manifestations of the study groups;

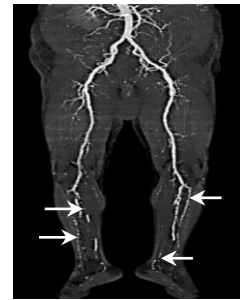
- Thirty-eight (100%) patients with diabetes mellitus; Doppler ultrasound study showed 24 (63%) patients with atherosclerotic stenosis (14 single and 10 multilevel stenosis) and 14 (37%) patients with complete occlusion (8 single, 6 multilevel occlusion) and Thirty-four (94%) patients presented with rest pain and ischemia; ultrasound study showed 18 (47%) patients with atherosclerotic stenosis (10 single, 8 multilevel stenosis) and 16 (42%) patients with complete occlusion (8 single, and 8 multilevel occlusion).
- Twenty-eight (74%) patients presented with claudication; ultrasound study showed 12 (32%) patients with atherosclerotic stenosis (6 single and 6 multilevel stenosis) and 16 (42%) patients with complete occlusion (10 single, and 6 multilevel occlusion).
- Two (5%) patients were presented with trauma; ultrasound study showed single complete occlusion in both patients (table 1), (figures 3,4,5,6).



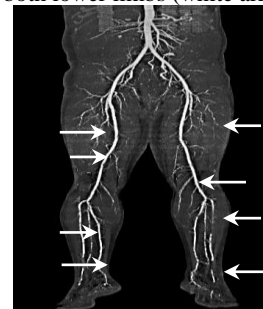
(Figure 3) Shows relation of manifestations with MDCTA results of the single and multiple steno-occlusive lesions of the lower limbs peripheral arteries.



(Figure 4) MDCTA for 30 yrs. patient with history of gunshot; The scanning showed complete cut and occlusion of superficial femoral artery of left lower limb (white arrows), stitching in skin was noted (red arrows).

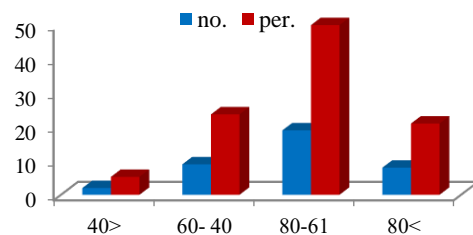


(Figure 5) MDCTA for 75 years old patient. Patient showing extensive atherosclerosis associated with multiple, complete and partial occlusion of anterior tibial, posterior tibial and peroneal arteries as well as multiple stenosis at lower level of superficial femoral arteries for both lower limbs (white arrows).



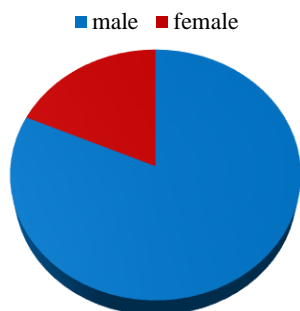
(Figure 6) MDCTA for 62 years old patient with typical multiple atherosclerotic complete occlusion and stenosis in peroneal, anterior and posterior tibial arteries of both lower limbs (white arrows).

The patients with steno-occlusive disease were divided into age groups. The age groups of patients were among 30 to 81 years. 19 (50%) patients with the disease were in age group 61-80 years, followed by 9 (14%) patients were in age group 41-60 years, 8 (21%) patients were in age group > 80 years and 2 (5.3%) patients were in age group < 40 years. The disease increased by increasing age (figure 7).



(Figure 7) Number and percentage of patients with lower limb peripheral arteries steno-occlusive lesion in age groups.

Out of thirty-eight patients were 31(81%) males and 7(19%) were females with lower limbs peripheral arteries steno-occlusive lesion. In this study showed the disease was highly affected males compared to females with ratio of 4.5:1 (figure 8).



(Figure 8) male to female ratio was 4.5:1.

### DISCUSSION

Lower extremities' peripheral vascular disease diagnosis usually undergoes with many radiological tools include ultrasonography (USG), digital subtraction angiography (DSA), MR angiography and MDCT angiography. MDCTA demonstrated features including size and extent of the vascular lesions and it could detect all the lesions in aorto-iliac segment very well as gas interfere the detection of cases on USG. DSA is invasive tool and has some morbidity whereas MDCTA was superior to DSA in visualization of peripheral territories<sup>(6,14)</sup>. MDCTA was the modality of quick examination compared to MRA and DSA. MRA for patients with pacemaker or definite other implants is contra-indicated and can't be used in uncooperative patients. MDCTA of peripheral vascular disease was the preferred imaging modalities to characterize all types of the detected vascular lesions including steno-occlusive disease. It was increasingly used for minimally invasive imaging of vascular territories<sup>(9,13)</sup> in patients who presented with clinical data (claudication, D.M., ischemia, trauma and all complaints related to arterial disease).

MDCTA underwent for all referred patients to evaluate PAD of lower limbs who presented with D.M, claudication, rest pain with ischemia and trauma and showed accurate imaging of the lower extremities' arteries.

The study showed steno-occlusive disease of the lower extremities in the examined patients was with mean age of 61 years and increased by increasing age-groups, nearly equal to the compared international study that showed the lower limbs arterial occlusion was secondary to atherosclerotic stenosis began after the age of 50 years<sup>(2)</sup>, (figure 7).

Our study showed the lower limbs peripheral arterial steno-occlusive disease affects males more than females. This result was not different significantly compared with the other studies<sup>(17)</sup>, (figure 8).

MDCTA examination in the study showed all cases with atherosclerosis as well as most of these cases were arterial stenosis (single level stenosis or multi-

level stenosis) or complete arterial occlusion (single complete occlusion or multilevel complete occlusion) similar to the other international studies showing the atherosclerosis was the most form of the peripheral arterial disease<sup>(4)</sup>, (figure 3).

MDCTA for evaluation of lower extremities arteries in our study all the examined (thirty-eight) patients presented with long-standing diabetes mellitus accompanied with or without other clinical manifestations such as claudication, rest pain with ischemia and trauma were highly associated with lower limbs arterial steno-occlusive disease. One amputated thigh of a patient with diabetes mellitus. The other presented patients were with different stages of ischemia (table 1).

(Table 1) Shows the correlation of clinical manifestation of the presented patients with various levels of steno-occlusive lesions.

	One level complete occlusion	Multilevel complete occlusion	One level stenosis	Multilevel stenosis	Total
Claudication	10	6	6	6	28
D.M.	8	6	14	10	38
Ischemia	8	8	10	8	34
Trauma	2				2

The other international studies showed participants who had PAD and diabetes mellitus was associated with great severity and diffuse peripheral arterial disease relative to non-diabetics and with greater risk of mortality and impaired quality life. The claudication in diabetic patient was twice of non-diabetic patients and PAD was more aggressive in patients with D.M. than in non-diabetic patients<sup>(4,18,19,20)</sup>.

Two patients presented with trauma. Two of those patients came with gunshot at his left thigh. MDCTA showed cutting popliteal artery. Emergency operative procedure performed for patient in the hospital before scanning.

### CONCLUSION

Peripheral vascular disease and atherosclerosis diagnosis became easy with MDCTA. This diagnostic technique was simple and non-invasive to evaluate the vascular study and now it is one of the methods of choice for diagnosis of vascular disease. The atherosclerotic steno-occlusive lesion increased by increasing age and appeared more in males than females. MDCTA was highly effective in diagnosis of PAD that mostly accompanied with diabetes mellitus with or without other clinical manifestations.

### ACKNOWLEDGMENTS

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## STUDYING THE PREVALENCE OF QUINOLONES AND AMINOGLYCOSIDES RESISTANCE PROFILES AND TRANSMISSION OF AAC (6')-IB GENE FROM CHICKEN TO CONTACT HUMAN

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### ABSTRACT

The spread of resistant Enterobacteriaceae to antibiotics in chicken farms constitute a reservoir of resistant genes that could be easily transmitted to contact human. So, this study conducted to investigate the prevalence of ciprofloxacin and gentamycin resistance profiles and the frequency of aac (6')-Ib gene in chicken farms in Misurata city and living population in the vicinity of these farms. (135) cloacae swab from chicken and (107) urine samples from human living in these farms were collected. Isolation and identification of different Enterobacteriaceae strains is performed in antibiotic resistance profiles against ciprofloxacin and gentamycin antibiotics. DNA extracted from each sample and further screening for aac (6')-Ib-cr in ciprofloxacin and/or gentamycin resistant isolates by using of using PCR technique. Our results showed that 88.8% and 93.4% of chicken and human isolates confirmed to be Enterobacteriaceae respectively. There were non-significant association between the source of Enterobacteriaceae isolates and quinolones or aminoglycosides resistance profiles. The screening of the genetic determinant of gentamycin and ciprofloxacin resistance aac (6')-Ib-cr, revealed an impressive proximity between the frequency of this gene within the chicken and human isolates (33.6%, 33 %) respectively. In conclusion quinolones and aminoglycosides resistance profiles and the frequency of aac (6')-Ib-cr between chicken and human were nearly comparable, that would suggest the possible transmission of this gene from chicken to human.

**KEY WORDS:** Antibiotic resistance, Quinolones, Aminoglycosides, Enterobacteriaceae.

### INTRODUCTION

Antimicrobial agents are widely used in food-producing animal farms such as chicken for prevention, treatment of animal diseases and also as growth promoters. The intensive use of antimicrobial agents has major concern due to the possibility of emergence and dissemination of the resistant genes to human through animals<sup>(1)</sup>. The workers in the farms, abattoirs, veterinarians, and their families are directly at high risk of infection with resistant bacteria due to close contact with animals<sup>(2)</sup>. This transmission does not constitute initially a major health threat for the population. However, workers and their families represent a pass channel of resistance genes to the hospital, and community environment. Moreover, the extra spread of these resistant genes to other pathogens is likely to be possible<sup>(3)</sup>. Many studies have highlighted the possible transmission of resistance from poultry to workers<sup>(4)</sup>.

Enterobacteriaceae spp. are important human pathogens that because hospital acquired infection such as urinary tract infection (UTI) and gastrointestinal infection<sup>(5)</sup>. The resistant Enterobacteriaceae have a major health concern in both pathogenic and commensal ones<sup>(6)</sup>. Many species of Enterobacteriaceae family cause UTI, which is the second cause of

community-onset infection<sup>(7)</sup>. 75-95% of all uncomplicated cystitis and pyelonephritis caused by E. coli which the most common cause of UTI<sup>(8)</sup>.

Quinolone antibiotics such as ciprofloxacin, ofloxacin and levofloxacin are used to treat wide range of gram-negative and gram-positive bacterial infections. They are used clinically because of proved bactericidal effect against most members of Enterobacteriaceae<sup>(9)</sup>.

Aminoglycosides have antimicrobial activity against gram-negative and gram-positive bacteria, therefore aminoglycosides are considered a broad-spectrum antibiotic that are active against Enterobacteriaceae and Pseudomonas<sup>(10)</sup>.

The use of fluoroquinolones, aminoglycosides, and third generation cephalosporins in animal farms and to treat human infection is hazardous those antibiotics are already used to treat human infections<sup>(11)</sup>.

AAC (6') genes have clinical importance because they can modify a number of aminoglycosides antibiotic, including amikacin, gentamicin, and tobramycin. The aac (6')-I type responsible for resistance to amikacin by acetylation of the drug, while the aac (6')-II type acetylates gentamicin<sup>(12-13)</sup>. A recent mechanism of quinolones resistance is correlated to plasmid mediated quinolone resistant genes (aac(6')-Ib-cr, qnrA, qnrB, qnrS, and qepA)<sup>(14)</sup>. The first gene aac (6')-Ib-cr is a variant of aminoglycoside acetyltransferase which reduces fluoroquinolones activity by binding of acetyl group to the drug. AAC (6')-Ib-cr is common gene that causes resistance to both aminoglycoside and fluoroquinolone antibiotics<sup>(15)</sup>.

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Usually, plasmid mediated quinolone resistance (PMQR) is correlated with extended spectrum beta-lactamases (ESBLs) and/or aminoglycosides resistant genes on the same plasmid, and transfer of multidrug resistant plasmids between Enterobacteriaceae strains would negatively affect the empirical treatment of complicated urinary tract infections<sup>(16)</sup>.

So, this work is conducted to Investigate the prevalence of ciprofloxacin and gentamycin resistance profiles and the frequency of aac(6')-Ib-cr gene in chicken farms in Misurata city and living population in the vicinity of these farms.

## METHODS

### Bacterial isolates:

In this study, 135 bacterial isolates were collected from chicken cloacae at 20 different chicken farms located in Misurata, sterile cotton swabs were used to collect fecal samples from chicken cloacae then swabs are immediately transferred to sterile collection containers. A 107 Clean-Catch midstream urine samples were collected in a sterile tube (4-5 ml) from people in contact with chicken farms. At the bioresearch and consultancies center, Misurata University, samples were cultured on MaCconkey agar (OXOID, England) then incubated for 18-24hr at 35-37°C. Colonies may appear pink (lactose fermenting) or colorless (lactose non-fermenting). Size and shape of colonies vary with individual species. Suspected colonies are identified using the APIE20: (bioMérieux®, France)<sup>(17)</sup>.

### Antibiotic susceptibility testing:

(Table 1) Primers used for detection of aac(6')-Ib gene and RAPD typing, annealing temperatures (Ta), and expected product sizes.

Primers	Sequence (5'-3')	Target gene	T <sub>a</sub>	Product size	Reference
Aac (6')-Ib-F aac (6')-Ib-R	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	aac(6')-Ib	54°C	482 bp	18

### Statistical analysis:

Data were presented in frequency and percentage. Chi square test using SPSS/21 was done for these data where the level of significance ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

An inevitable side effect of the use of antibiotics is the emergence and dissemination of resistant bacteria. Most studies show that after the introduction of an antibiotic not only increase the level of resistance in pathogenic bacteria, but also in commensal bacteria. Commensal bacteria constitute a reservoir of resistant genes for (potentially) pathogenic bacteria. Their level of resistance is considered to be a good indicator for selection pressure by antibiotic use and for resistance problems to be expected in pathogens<sup>(21)</sup>. Antibiotic resistance in bacteria from the family Enterobacteriaceae is an important indicator of the emergence of resistant bacterial strains in the community<sup>(22)</sup>. Farm and slaughterhouse workers with resistant bacteria through close contact with colonized or infected animals. Although this limited

Antibiotic sensitivity test for chicken and human samples is performed by using Mueller-Hinton agar according to Kirby-Bauer Disc Diffusion method following clinical and laboratory standards institute (CLSI) guidelines. The antibiotics included in this study are ciprofloxacin 5µg and gentamycin 10µg (Bioanalyse®, Turkey)<sup>(18)</sup>. The interpretation and results reporting of the categories of susceptible, intermediate or resistant was also based on the CLSI guidelines<sup>(19)</sup>.

### Bacterial DNA extraction:

The isolates are streaked on nutrient agar and incubated for 14-16 hr at 37° C. A single colony was picked up from the media plate and inoculated to 5 ml liquid culture media, then incubated overnight at 37° C. Genomic DNA was then extracted at the bioresearch and consultancies center, Misurata University, using the G-spin™ Total DNA Extraction Kit (INTRON Biotechnology, Korea) according to the manufacturer's recommendation.

### Detection of antibiotic-resistance genes:

This procedure was performed in animal health institute, Cairo, Egypt, where Polymerase chain reaction (PCR) identification of aminoglycoside and quinolone resistance genes aac (6')-Ib was performed as described in previous study<sup>(20)</sup>.

The PCR conditions involved an initial denaturation for 3 min at 95 °C followed by 30 cycles of (95 °C for 30 s, specific annealing temperature for 1 min, and extension at 72 °C for 30 s) followed by a final extension at 72 °C for 5 min. Sequences of the resistance-genes primers used in the study and their annealing temperatures are shown in (table 1).

transmission does not initially appear to pose a population-level health threat. Occupational workers and their families provide a conduit for the entry of resistance genes to the community and hospital environments, where further spread into pathogens is possible<sup>(23,24)</sup>. We performed this study in order to investigate the prevalence of quinolone and aminoglycosides resistance pattern and the frequency of aac (6')-Ib-cr gene in commensal and pathogenic isolates from chicken farms and urine of contact human (either farm workers or living population near to farms). In this study, Enterobacteriaceae species isolates accounts for (88.8%) in chicken and (93.4%) In human isolates.

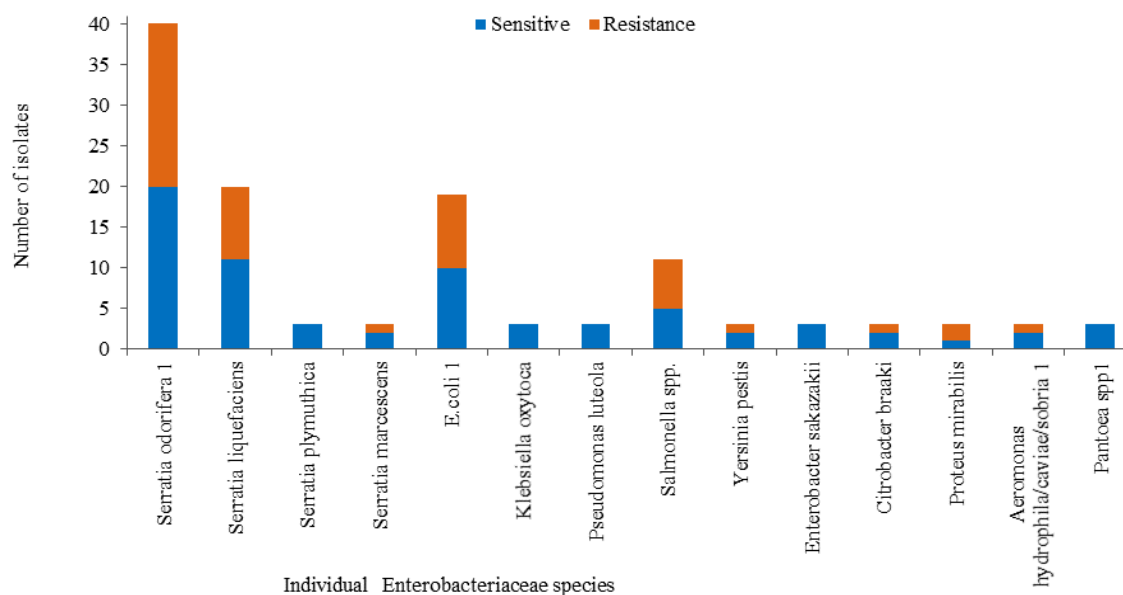
Different Enterobacteriaceae strains were identified from chicken including; E. coli 19/120 (15.8%), Salmonella spp. 11/120 (9.1%) and Yersinia pestis 3/120 (2.5%) pathogenic bacteria and represent a zoonotic threat to contact human. There were other Enterobacteriaceae isolates from chicken that would be opportunistic pathogen such as Serratia spp. 66/120 (55%), and 3/120 (2.5%) for Pseudomonas luteola, Klebsiel-

la oxytoca, Citrobacter brakii, Enterobacter sakazakii, Proteus mirabilis and Pantoea spp. The frequency of individual Enterobacteriaceae spp. of chicken isolates

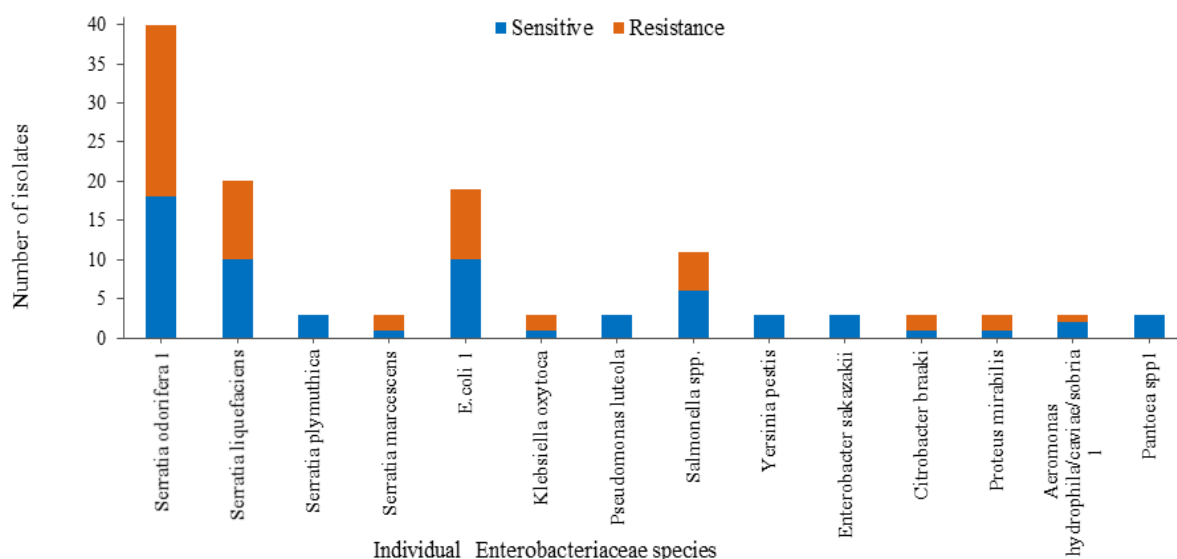
and their resistance pattern are shown in (figure 1,2) and (table 2).

(Table 2) The frequency and antibiotics (ciprofloxacin & gentamycin) resistance profiles of individual Enterobacteriaceae in chicken and human isolates.

Name of Enterobacteriaceae	Source of Enterobacteriaceae	No. % of Enterobacteriaceae isolates	Ciprofloxacin		Gentamycin	
			R	S	R	S
Serratia odorifera 1	Human	12/100 (12)	5/12 (41.7)	7/12 (58.33)	6/12 (50)	6/12 (50)
	Chicken	40/120 (33.3)	20/40 (50)	20/40 (50)	22/40 (55)	18/40 (45)
Serratia liquefaciens	Human	11/100 (11)	3/11 (27.3)	8/11 (72.7)	6/11 (54.5)	5/11 (45.5)
	Chicken	20/120 (16.6)	9/20 (45)	11/20 (55)	10/20 (50)	10/20 (50)
Serratia plymuthica	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	0/3 (0)	3/3 (100)
Serratia marcescens	Human	3/100 (3)	1/3 (33.3)	2/3 (66.6)	2/3 (66.6)	1/3 (33.3)
	Chicken	3/120 (2.5)	1/3 (33.3)	2/3 (66.6)	2/3 (66.6)	1/3 (33.3)
E. coli 1	Human	20/100 (20)	8/20 (40)	12/20 (60)	9/20 (45)	11/20 (55)
	Chicken	19/120 (15.8)	9/19 (47.4)	10/19 (52.6)	9/19 (47.4)	10/19 (52.6)
Klebsiella pneumonia	Human	17/100 (17)	7/17 (41.2)	10/17 (58.8)	9/17 (52.9)	8/17 (47.1)
	Chicken	-	-	-	-	-
Klebsiella oxytoca	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	2/3 (66.6)	1/3 (33.3)
Pseudomonas luteola	Human	6/100 (6)	4/6 (66.6)	2/6 (33.3)	2/6 (33.3)	4/6 (66.6)
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	0/3 (0)	3/3 (100)
Pseudomonas fluorescens/putida	Human	3/100 (3)	1/3 (33.3)	2/3 (66.6)	0/3 (0)	3/3 (100)
	Chicken	-	-	-	-	-
Salmonella spp.	Human	3/100 (3)	1/3 (33.3)	2/6 (66.6)	2/3 (66.6)	1/3 (33.3)
	Chicken	11/120 (9.1)	6/11 (54.5)	5/11 (45.5)	5/11 (45.5)	6/11 (54.5)
Yersinia pestis	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	1/3 (33.3)	2/3 (66.6)	0/3 (0)	3/3 (100)
Enterobacter sakazakii	Human	3/100 (3)	1/ (33.3)	2/3 (66.6)	2/3 (66.6)	1/ (33.3)
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	0/3 (0)	3/3 (100)
Enterobacter cloacae	Human	6/100 (6)	2/6 (33.3)	4/6 (66.6)	2/6 (33.3)	4/6 (66.6)
	Chicken	-	-	-	-	-
Citrobacter braaki	Human	3/100 (3)	2/3 (66.6)	1/3 (33.3)	0/3 (0)	3/3 (100)
	Chicken	3/120 (2.5)	1/3 (33.3)	2/3 (66.6)	2/3 (66.6)	1/3 (33.3)
Proteus mirabilis	Human	3/100 (3)	1/3 (33.3)	2/3 (66.6)	1/3 (33.3)	2/3 (66.6)
	Chicken	3/120 (2.5)	2/3 (66.6)	1/3 (33.3)	2/3 (66.6)	1/3 (33.3)
Rauteella ornithinolytica	Human	7/100 (7)	3/7 (42.3)	4/7 (57.7)	2/7 (28.6)	5/7 (71.4)
	Chicken	-	-	-	-	-
Acintobacter baumannii/calcoaceticus	Human	3/100 (3)	1/3 (33.3)	2/3 (66.6)	0/3 (0)	3/3 (100)
	Chicken	-	-	-	-	-
Aeromonas hydrophila/caviae/sobria 1	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	1/3 (33.3)	2/3 (66.6)	1/3 (33.3)	2/3 (66.6)
Pantoea spp1	Human	-	-	-	-	-
	Chicken	3/120 (2.5)	0/3 (0)	3/3 (100)	0/3 (0)	3/3 (100)



(Figure 1) Isolation, identification and ciprofloxacin resistance profile of chicken isolates.



(Figure 2) Isolation, identification and gentamycin resistance profile of chicken isolates.

Kilonzo-Nthenge et al.<sup>(25)</sup> studied the occurrence of Enterobacteriaceae in retail meats, Out of 281 bacteria isolates from raw meat samples, (12.1%) were identified as *E. coli*, *Morganella morgana* (1.1%), *Vibrio parahemolyticus* (0.4%), *Yersinia enterocolitica* (0.4%), *Salmonella* spp. (5.7%), *Proteus mirabilis* (1.1%), *Enterobacter aerogenes* (6.4%), *Klebsiella oxytoca* (27.4%), *Citrobacter freundii* (1.7%) *Hafnia alvei* (11.4%) *Serratia* spp. (14.3%) *Enterobacter aerogenes* (6.4%), *Kluyvera* spp. (5.6%), and *Pantoea* spp. (3.6%). The occurrence of *Klebsiella oxytoca* in retail meats was the highest among all other pathogens. While, Yulistiani et al.<sup>(19)</sup> investigated the prevalence of antibiotic-resistant Enterobacteriaceae isolated from chicken meat contaminated during evisceration and sold at traditional markets in Surabaya Indonesia. In all 203 isolates; *Salmonella* spp. (41.7%), *E.coli* (26.1%), *Citrobacter* spp. (10.8),

*Klebsiella* spp. (6.4%), *Proteus* spp. (11.8%), *Yersinia* spp. (7.3), *Enterobacter* spp. (3.4%) and *Serratia* spp. (2.9%) were identified. These results were high consistent with our results except for *Serratia* spp. In our study the prevalence of *Serratia* spp. is high in chicken isolates which are divided into four spp.; *Serratia odorifera* 1 (33.3%), *Serratia liquefaciens* (16.6%), *Serratia plymuthica* (2.5%), and *Serratia marcescens* (2.5%). *Serratia marcescens* is serious pathogen capable of causing important infections in human and animals such as (UTIs) and pneumonia, which becomes highly prevalent and shows a multi-drug resistance profile<sup>(26)</sup>.

After investigation of chicken isolates, we found that 41.7% of Enterobacteriaceae were resistant to ciprofloxacin and 45.8% resistant to gentamycin as shown in (table 3).

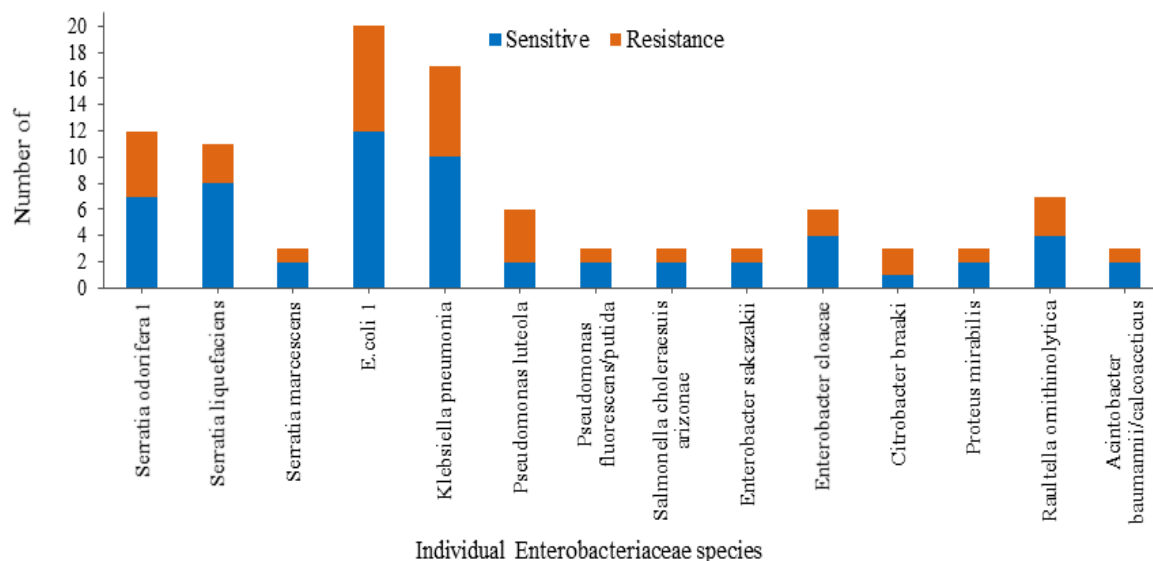
(Table 3) Antibiotic resistance profile of Enterobacteriaceae family against ciprofloxacin and gentamycin antibiotics in chicken and human isolates.

Source of Enterobacteriaceae	Ciprofloxacin		P- value	Gentamycin		P-value
	R	S		R	S	
Human	40/100 (40)	60/100 (60)	0.802	43//100 (43)	57/100 (57)	0.67
Chicken	50/120 (41.7)	70/120 (58.3)		55/100 (45.8)	65/120 (54.2)	

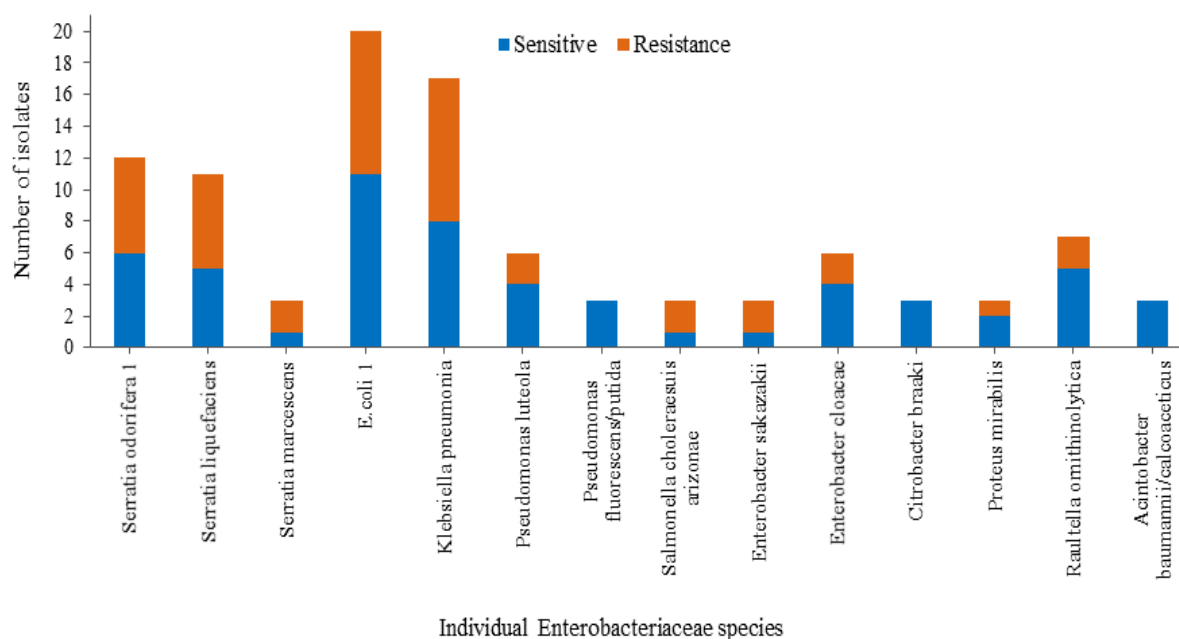
Kilonzo-Nthenge et al.<sup>(25)</sup> and Yulistiani et al.<sup>(22)</sup> found lower resistance rates of Enterobacteriaceae against gentamycin (9.6%) and (5.91%) isolated from retail meat respectively. previous studies reported that ciprofloxacin resistance in chicken meat isolates were (12.5%) and (0%) respectively<sup>(27,28)</sup>.

The isolated Enterobacteriaceae strains from urine samples of accompanying human were; *E.coli* (20%), *Klebsiella pneumonia* (17%), *Serratia odorifera* 1 (12%), *Serratia liquefaciens* (11%), *Raultella ornithi-*

*nolytica* (7%), *Pseudomonas luteola* and *Enterobacter cloacae* (6%), and others were lower percentage as; *Serratia marcescens*, *Pseudomonas fluorescens/putida*, *Salmonella* spp., *Enterobacter sakazakii*, *Citrobacter braaki*, *Proteus mirabilis*, *Acinetobacter baumannii/calcoaceticus*. The frequency of individual Enterobacteriaceae spp. of human isolates and their resistance pattern shown in (figure 3,4) and (table 2).



(Figure 3) Isolation, identification and ciprofloxacin resistance profile of human isolates.



(Figure 4) Isolation, identification and gentamycin resistance profile of human isolates.

The present study conducted in Misurata city in Libya showed that higher prevalence of *E. coli*, *K. pneumonia*, *E. cloacae* and *Serratia marcescens* than that conducted in Tripoli city in Libya<sup>(29)</sup>. While Yang et al.<sup>(30)</sup> reported that Enterobacteriaceae composed of 88.5% of the total isolates, with exceeding rate of *E. coli* (63.2%) and *K. pneumoniae* (12.2%).

Concerning human isolates about 40% and 43% exhibited resistance against ciprofloxacin and gentamycin respectively as presented in (table 3).

Tobgi et al.<sup>(31)</sup> tested the susceptibility of *E. coli* to antibiotics. 148 *E. coli* isolated from urine specimens of outpatients attending Al-Jalla Hospital in Benghazi. They reported that 18% of isolates were resistant to gentamicin. Ghenghesh et al.<sup>(32)</sup> found 7% resistance to gentamicin of 538 *E. coli* isolates from patients with UTIs in Tripoli.

M. I. Issack et al.<sup>(33)</sup> observed the presence of high rate of resistance to fluoroquinolone (26.4% to ciprofloxacin). In a survey conducted in India between 2010 and 2014, they found an increase resistance rate in Enterobacteriaceae isolated from hospitalized patients against ciprofloxacin (39.2-56.1%)<sup>(33,34)</sup>.

Statistical analysis of the antibiotic resistance profiles against ciprofloxacin and gentamycin in both chicken and human revealed no association ( $P > 0.05$ ) between the source of isolates and the pattern of resistance. In other words, we can say that the rates of resistance against ciprofloxacin and gentamycin in the two species were nearly comparable (table 4). These results convey a proof of the possible transmission of resistant gene between chicken and contact human.

**(Table 4)** Antibiotic resistance profiles of the common Enterobacteriaceae individual spp. in chicken and human isolates

Name of Enterobacteriaceae	Source of Enterobacteriaceae	Ciprofloxacin			Gentamycin		
		R	S	P-value	R	S	P-value
Serratia species	Human	9/26 (34.6)	17/26 (65.4)	0.343	14/26 (53.8)	12/26 (46.2)	0.840
	Chicken	30/66 (45.4)	36/66 (54.5)		34/66 (51.5)	32/66 (48.5)	
Serratia marcescens	Human	1/3 (33.3)	2/3 (66.6)	1.000	2/3 (66.6)	1/3 (33.3)	1.000
	Chicken	1/3 (33.3)	2/3 (66.6)		2/3 (66.6)	1/3 (33.3)	
E. coli 1	Human	8/20 (40)	12/20 (60)	0.643	9/20 (45)	11/20 (55)	0.882
	Chicken	9/19 (47.4)	10/19 (52.6)		9/19 (47.4)	10/19 (52.6)	
Salmonella species	Human	1/3 (33.3)	2/6 (66.6)	0.515	2/3 (66.6)	1/3 (33.3)	0.515
	Chicken	6/11 (54.5)	5/11 (45.5)		5/11 (45.5)	6/11 (54.5)	
Citrobacter braaki	Human	2/3 (66.6)	1/3 (33.3)	0.414	0/3 (0)	3/3 (100)	0.083
	Chicken	1/3 (33.3)	2/3 (66.6)		2/3 (66.6)	1/3 (33.3)	
Proteus mirabilis	Human	1/3 (33.3)	2/3 (66.6)	0.414	1/3 (33.3)	2/3 (66.6)	0.414
	Chicken	2/3 (66.6)	1/3 (33.3)		2/3 (66.6)	1/3 (33.3)	

The screening of the genetic determinant of gentamycin and ciprofloxacin resistance, aac (6') Ib-cr, revealed an impressive proximity between the frequency of this gene within the chicken and human isolates (28.33%, 33 %) respectively that supposes prodigious evidence for the transmission of resistance (table 5).

**(Table 5)** Prevalence of aac (6')-Ib-cr gene in chicken and human.

Source of Enterobacteriaceae	aac(6')-Ib-cr (No)	aac(6')-Ib-cr (%)
Chicken	34	28.3
Human	33	33

The aac (6')-Ib-cr is a plasmid-mediated quinolone resistance (PMQR) gene embedded within a gene cassette, most often within an integron. It confers resistance to both quinolone and aminoglycoside<sup>(35)</sup>. Agabou et al.<sup>(36)</sup> found that commensal E. coli isolates collected from chickens, their farmers, and patients in the Constantine region (North-east Algeria) were analyzed for plasmid mediated quinolone resistance (PMQR) gene contents types. A high prevalence of resistance to fluoroquinolone (51.4 % to ciprofloxacin) was recorded in avian isolates. Of these, (22.2 %) carried the aac 6')-Ib-cr gene, While seventy pathogenic isolates were resistant to fluoroquinolone, with aac(6')-Ib-cr present in (72.8 %). Park et al.<sup>(37)</sup> isolated 313 human Enterobacteriaceae isolates from the United States, aac(6')-Ib was present in 50.5% of isolates, and of these, 28% carried the (cr) variant responsible for low-level ciprofloxacin resistance. Yang et al.<sup>(30)</sup> showed that aac(6')-Ib-cr was present in 17.0% of the E.coli human isolates, and 7.9% of the isolates carried both the qnr and the aac(6')-Ib-cr genes.

Resistance to aminoglycosides may be observed at several levels. High-level resistance is generally associated with chemical modifications of the aminoglycoside by enzymes<sup>(38)</sup>. Studies of aminoglycoside-resistant organisms from different countries have shown that the resistance mechanisms are different

and may be a country/area specific resistance<sup>(39)</sup>. A surveillance study conducted in European countries on Enterobacteriaceae showed that resistance against gentamicin was between (2-13%) and the found gene responsible for this resistance were aac (3')-IIa, followed by aac (6')-Ib-cr<sup>(40)</sup>. AAC enzymes are important because they are among the few that confer resistance to aminoglycosides and quinolones Schmitz et al.<sup>(43)</sup>.

Miró et al.<sup>(41)</sup> studied the characterization of aminoglycoside-modifying enzymes in Enterobacteriaceae clinical Strains and found the most frequent gene was aph (3')-Ia (13.9%) and aac (3)-IIa (12.4%), followed by aac (6')-Ib (4.2%), and among the 14 aac (6')-Ib eight showed the (cr) variant (57.1%)

## CONCLUSION

In this study, it is concluded that Enterobacteriaceae isolates from chicken and human showed no difference in their resistance pattern against ciprofloxacin and gentamicin antibiotics. The prevalence of aac (6')-Ib gene within chicken and human provide a possible proof of transmission of this resistance gene between the two species.

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# PHENOTYPIC AND GENOTYPIC INVESTIGATION OF OXA23 AND OXA51 CARBAPENEMASES PRODUCING ACINETOBACTER BAUMANNII IN TRIPOLI HOSPITALS

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## ABSTRACT

*Acinetobacter baumannii* is an opportunistic pathogen causing various nosocomial infections. The aim of this study was to characterize the molecular support of carbapenem-resistant *A. baumannii* clinical isolates recovered from four hospitals in Tripoli, Libya. Bacterial isolates were identified and antibiotic susceptibility testing was performed using automated system. Carbapenem resistance determinants were studied phenotypically using two different techniques: E-test; chromogenic culture media. Polymerase chain reaction (PCR) amplification was used to determine the presence of *bla*<sub>OXA23</sub> and *bla*<sub>OXA51</sub> genes among isolates. A total of 119 isolates were characterized, overall the resistance prevalence was extremely high for aminoglycosides (79-96.6%), fluoroquinolones (94-96%), cephalosporins (96.6-100%) and carbapenemes (93.2-100%), all isolates were susceptible to colistin. In addition, 97.5% of isolates were identified as multidrug resistance (MDR). Varying degree of phenotypic detection of carbapenemes was determined; highest levels of carbapenemes were detected using chromogenic media (76.5%) compared with E-test (45.4 %). The carbapenem resistance-encoding genes detected were *bla*<sub>OXA23</sub> (84%) and *bla*<sub>OXA51</sub> (73.1%); the highest occurrence of *bla*<sub>OXA23</sub> was demonstrated in Tripoli's Central Hospital (5/5; 100%) then in Tripoli Medical Center (44/51; 86.27%). The co-occurrence of these genes was demonstrated in (75/119; 63%) showing dissemination of carbapenemes resistance MDR *A. baumannii* in hospitals. This study shows that the high prevalence of OXA-23 contribute to antibiotic resistance in Libyan hospitals and represents the high incidence of the association of these two carbapenemases in an autochthonous MDR *A. baumannii* isolated from patients in Libya, indicating that there is a longstanding infection control problem in these hospitals.

**KEY WORDS:** *bla*<sub>OXA23</sub>, *bla*<sub>OXA51</sub>, *A. baumannii*, Tripoli, Libya.

## INTRODUCTION

*A. baumannii* is an opportunistic pathogen mainly involved in healthcare-associated infections, with increased mortality and morbidity<sup>(1)</sup>. It is associated with a wide range of clinical complications, such as pneumonia, septicemia, urinary tract infection, wound infection and meningitis, particularly in immunocompromised patients<sup>(2)</sup>. The serious concern associated with this bacterium is the increasing prevalence of multidrug resistant isolates, especially carbapenem resistant ones. Outbreaks of carbapenem resistant *A. baumannii* strains have been documented in diverse geographical areas including Europe, South America and Asia<sup>(3-5)</sup>, but little information is available from North Africa<sup>(6,7)</sup>. In Libya, dissemination of carbapenemases, such as the *bla*<sub>OXA-23</sub>-like and *bla*<sub>OXA-24</sub>-like genes, among *A. baumannii* isolates has been reported previously<sup>(8)</sup>.

Carbapenem resistance in *Acinetobacter* species is most commonly caused by the production of OXA-type carbapenemases<sup>(9)</sup>. The OXA-type carbapenemases comprise four broad groups: *bla*<sub>OXA-23</sub>-like, *bla*<sub>OXA-40</sub>-like, *bla*<sub>OXA-58</sub>-like and an intrinsic *bla*<sub>OXA-51</sub>-like<sup>(10,11)</sup>. In Libya, limited numbers of epidemiological studies concerning *A. baumannii* have been reported<sup>(7,8)</sup>. Such information

is important in guiding clinicians to select the best alternative drug(s) to treat serious infections associated with carbapenem resistant *A. baumannii*. The aim of this study was to characterize *A. baumannii* molecular epidemiology in Tripoli-Libya.

## MATERIALS AND METHODS

### Identification and antibiotic susceptibility testing of isolates

During 2013-2014, Specimens were collected from different anatomical sites including (urine, stool, sputum, cerebro spinal fluid, blood), swabs (wound exudates, ear, throat, rectal, axilla, nasal), endotracheal tube tip, central line tube, urine catheter, excretion in naso gastric tube. All specimens were taken as part of the clinical workup was included in this laboratory-based surveillance study. Demographic data, age, gender of the patients, in/out patients, department, and type of specimens were recorded from four major teaching hospitals: Tripoli Medical Centre (TMC); Tripoli Pediatric Hospital (TPH); Burn and Plastic Surgery Hospital (BPSH) and Tripoli Central Hospital (TCH). All isolates were identified to the species level and tested for their susceptibility to a variety of antimicrobial agents by the BD Phoenix Automated Microbiology System (USA) according to the manufacturer's instructions. *A. baumannii* isolates that showed resistance to at least three classes of antibiotics such as fluoroquinolones, aminoglycosides, and cephalosporins were defined as multidrug resistant (MDR) in accordance to the definitions provided by Magiorakos and colleagues<sup>(12)</sup>.

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### Phenotypic detection of carbapenem-hydrolysing oxacillinases

Carbapenem resistance determinants were studied phenotypically using two different techniques: chromogenic culture media, this screening medium (Chromatic CRE) used for detection carbapenem-resistant *Enterobacteriaceae* and non-fermentative Gram negative bacilli (Liofilchem, Italy) and E-test (Liofilchem, Italy) according to manufacturer's instructions and as previously described<sup>(13)</sup>.

*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as controls for susceptibility testing. In this investigation, specimens were collected under approved ethical standards and the study was reviewed and approved by the Faculty of Pharmacy, University of Tripoli and hospitals participating in this study.

### Molecular detection of *bla*<sub>OXA23</sub> and *bla*<sub>OXA51</sub> genes

Polymerase chain reaction (PCR) amplification was used to determine the presence of carbapenem-hydrolysing oxacillinases *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-51</sub> genes among isolates. The primers used for PCR amplification of the carbapenemase genes are listed in (table 1).

**(Table 1)** Primers used in the amplification of selected carbapenemase genes

Name	Nucleotide sequence (5' → 3')	Product size (bp)	Location
OXA-23-like	F- GATGTGTCATAGTATTCGTCGT R- TCACAACAACAAAAGCAC-TGT	1064	<i>bla</i> <sub>OXA-23</sub>
OXA-51-like	F- TAATGCTTTGATCGGCCTTG R- TGGATTGCACTTCATCTTGG	353	<i>bla</i> <sub>OXA-51</sub>

All isolates were screened for the presence of genes encoding *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-51</sub> by PCR using previously reported primers<sup>14</sup>. The plasmids were isolated using the QIAGEN Plasmid Mini Kit (QIAGEN, Valencia, CA), according to the manufacturer's instructions. The reaction mixture contained a total of 25 µl: 5 µl of 5X Red Load Taq Mix composed of Taq Polymerase, 0.05 u/µl dNTPs (200 µM) (dATP, dCTP, dGTP, dTTP) reaction buffer with KCl and MgCl<sub>2</sub> (1.5 mM) red dye, gel loading buffer, stabilizers (Metabion, Martinsried- Germany); 0.5 µl of 10pmol/µl of each primer, 2-50ng of the extracted plasmid DNA. The thermal profile included one cycle of initial denaturation at 95°C for 2 min followed by 35 cycles at 95°C for 30 sec, annealing at 52°C for 30 sec, and extensions at 72°C for 45 sec. The PCR reaction was carried out with TC-412 thermocycler (Techne, Duxford, Cambridge, U.K.). Five µl of the PCR amplification products

were electrophoresed in agarose (2% m/v) containing 0.5 µg/mL ethidium bromide.

The amplified PCR products were visualized under UV light and electronically documented with a gel documentation system (MultiDoc-It Digital Imaging System UVP, Cambridge, UK). A 100bp DNA ladder (Metabion, Martinsried- Germany) was used as a molecular size marker.

### RESULTS

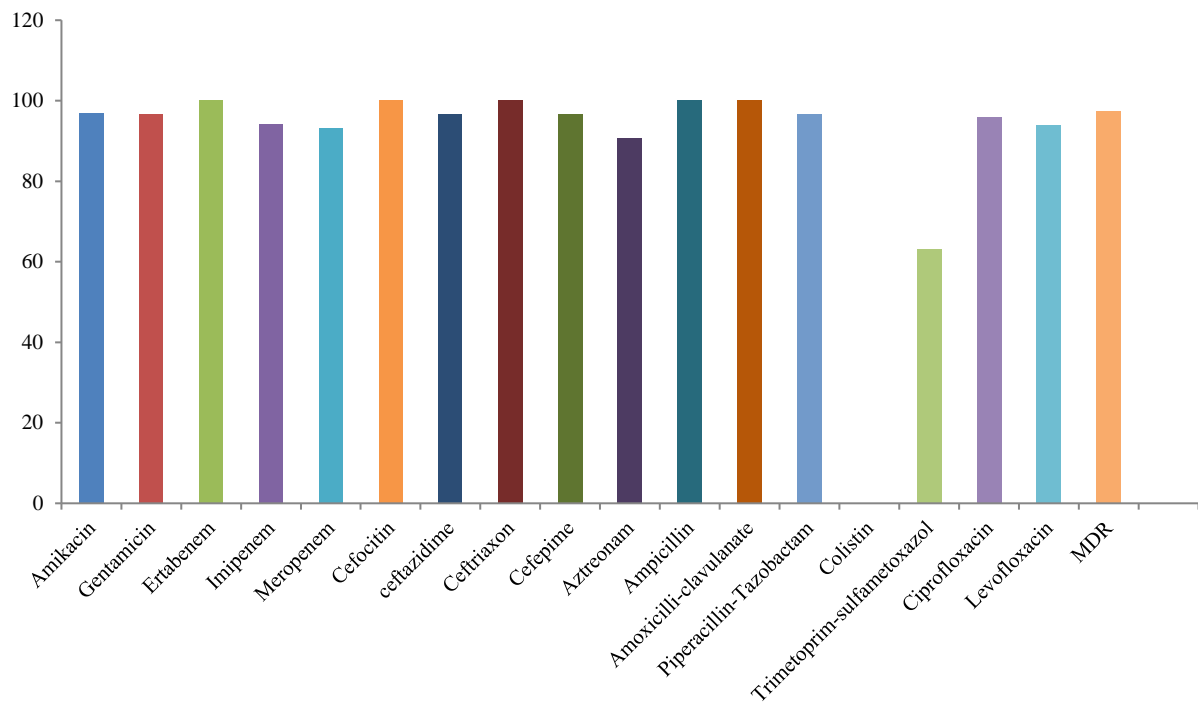
A total of 119 isolates *A. baumannii* isolates were characterized, the majority was isolated from patients at BPSH (57; 48%) and TMC (51; 42.8%) compared with other hospitals; TPH (6; 5%) and TCH (5; 4.2%). A 98.2% of strains were isolated from in-patients specimens primarily from burn wounds (61.5%) and less frequently from tips (ETT and central line etc.) (16.5%); blood (8.2%) and urine (5.5%). Most (85.3%) were obtained from patients hospitalized in ICUs (burn, neonatal, surgical etc.) and the remaining from patients housed in other hospital sectors.

Overall the resistance prevalence was extremely high for aminoglycosides (79-96.6%), fluoroquinolones (94-96%), cephalosporins (96.6-100%) and carbapenemes (93.2-100%), all isolates tested were susceptible to colistin. Over ninety percent of isolates showed resistance to imipenem and meropenem and exhibited minimum inhibitory concentration (MIC) >8µg/ml. In addition, 97.5% of isolates were identified as MDR (figure 1). Varying degree of phenotypic detection of carbapenemes was determined; highest levels of carbapenemes were detected using chromogenic media (76.5%) compared with E-test (45.4%) (Table 2).

**(Table 2)** Phenotypic and genotypic detection of carbapenem resistant *A. baumannii*

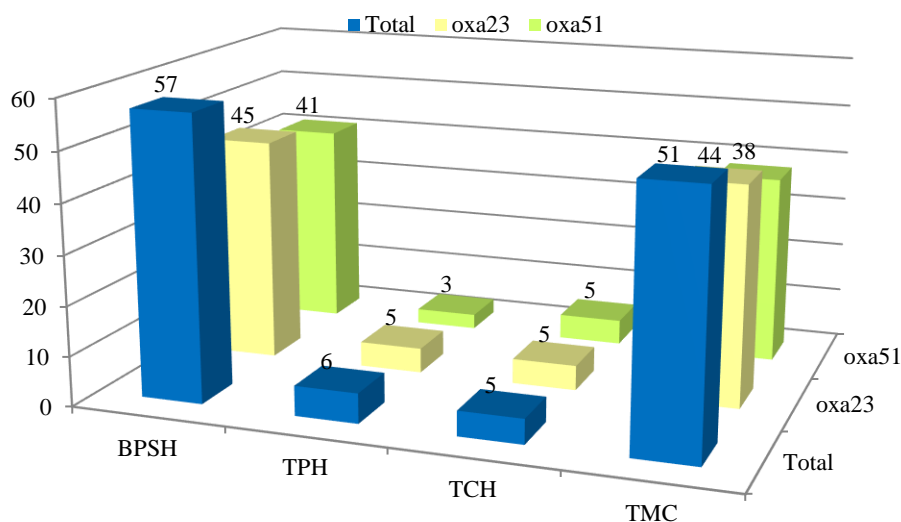
Isolate	phenotype		Genotype	
	E test MBL No (%)	Chromogen media No (%)	OXA-23 No (%)	OXA-51 No (%)
<i>A. Bau-mannii</i>	54 (45.4)	91 (75.5)	100 (84)	87 (73.1)

High level of carbapenem resistance-encoding genes were detected *bla*<sub>OXA23</sub> (84%) and *bla*<sub>OXA51</sub> (73.1%). *A. baumannii* harboring carbapenem resistance-encoding genes were mainly detected in ICUs (93/119; 78.1%); the highest was demonstrated in TCH (5/5; 100%) and BPSH (42/57; 73.7%) (Figure 2). The co-occurrence of *bla*<sub>OXA23</sub> and *bla*<sub>OXA51</sub> were demonstrated in (75/119; 63%) showing dissemination of carbapenemes resistance MDR *A. baumannii* in hospitals.



(Figure 1) Antibiotic resistance of *A. baumannii* isolated from different clinical specimens

Antibiotic	<i>A. Baumannii</i> = 119 (%)
Amikacin	94 (79)
Gentamicin	115 (96.6)
Ertapenem	119 (100)
Imipenem	112 (94.1)
Meropenem	111 (93.2)
Cefoxitin	119 (100)
Ceftazidime	115 (96.6)
Ceftriaxone	119 (100)
Cefepime	115 (96.6)
Ciprofloxacin	114 (96)
Levofloxacin	112 (94)
Amoxicillin-clavulanate	119 (100)
Piperacillin-Tazobactam	106 (89.1)
Colistin	0 (0)
Trimetoprim-sulfametoxazol	73 (61.3)
Aztreonam	119 (100)
Ampicillin	119 (100)
MDR	116 (97.5)
ESBL	115 (96.6)



(Figure 2) *A. baumannii* harbouring carbapenem resistance-encoding genes in four Tripoli hospitals.

### DISCUSSION

The widespread of carbapenem-resistant *A. baumannii* constitutes a global public health threat. Molecular characterization of mechanisms and epidemiology of MDR is a remarkable step to monitor its spreading and develop therapeutic strategies. Over ninety percent of *A. baumannii* strains collected from four hospitals in Tripoli were resistant to imipenem and meropenem, higher than that previously reported in Libya<sup>(15)</sup>. Carbapenem resistance has rapidly increased worldwide and prevalence of imipenem-resistant strains has reached 100% in some countries<sup>(16)</sup>.

All our imipenem-resistant strains were MDR, as commonly reported worldwide<sup>(17,18)</sup>, this is may be inherent to the accumulation of mutations selected by various antibiotics before introduction of carbapenems, and to the multiple mechanisms of carbapenem resistance in *A. baumannii* conferring simultaneous resistance to antibiotics of other classes<sup>(19)</sup>. Overall, MDR *Acinetobacter* strains remain susceptible to colistin. Besides imipenem, it also should be noted the very high prevalence (100%) of aztreonam resistance in our strains. This antibiotic, which normally is not or weakly hydrolyzed by OXA-carbapenemases types, constitutes a therapeutic solution in combination with a large broad spectrum serine beta-lactamases inhibitor<sup>(20)</sup>. In agreement with our study, the prevalence of trimethoprim/sulfamethoxazole resistance (61.3%) in *A. baumannii* is high in many geographic regions<sup>(21)</sup>. Trimethoprim resistance in *Acinetobacter* can be related to housekeeping *dfr* genes and to efflux systems<sup>(22,23)</sup>.

Carbapenem resistance was investigated by phenotypic and genotypic tests of all strains. Although few *A. baumannii* strains were found to be positive by phenotypic tests used in the study, while carbapenem resistance genes were not detected, this

might be adequately explained by the fact only two genes associated with carbapenems were investigated. Different studies have reported positive results by MBL phenotypic tests, but MBL resistance genes that could not be identified in *Acinetobacter* strains. Suggesting that carbapenem resistance genes, which are common in the region, should be investigated to evaluate the phenotypic test results correctly<sup>(24)</sup>.

Carbapenem resistance in *A. baumannii* is most often associated with class D  $\beta$ -lactamases (OXA-23-like, OXA-40-like and OXA-58-like). OXA-23-like is the most prevalent of carbapenemases with a global distribution and was described as cause of nosocomial outbreaks<sup>(25-27)</sup>. We found that OXA-23-type was the major (84%) carbapenemase mechanism responsible for the resistance phenotype. This finding is similar to data previously reported from the Gulf region<sup>(28,29)</sup> and our region (Egypt, Algeria and Tunis)<sup>(30-33)</sup>. Class D carbapenemases blaOXA-23 was identified in 72%, 72.5%, 67.02% and 90% of studied carbapenem-resistant *A. baumannii* strains in two Egyptian centres<sup>(34)</sup>, Saudi Arabia<sup>(35)</sup>, Algeria<sup>(19)</sup> and Lebanon<sup>(13)</sup>, respectively. Hammoudi and co-workers suggested that the predominance and dissemination of OXA-23 in Lebanon is consistent with the worldwide epidemiology of OXA-23 and with reports from neighboring countries<sup>(13)</sup>. It is frequently detected in isolates from Asia and Europe, and in most cases, it is found concomitantly with the blaOXA-51-like gene<sup>(36,37)</sup>. In this study, the co-occurrence of blaOXA23 and blaOXA51 gene was detected in 63%; 75/119 of isolates. Through molecular methods this study has indicated that 73.1% of the *A. baumannii* isolates contained the OXA-51 gene. Although it is clear that blaOXA-51-like genes are present in at least the vast majority of isolates of *A. baumannii*, there has been some debate as to whether they are present in all isolates of this species<sup>(38)</sup>.

### CONCLUSION

The high prevalence of OXA-23 and OXA-51 among *A. baumannii* contributes to antibiotic resistance in Libyan hospitals with a great potential for spread in ICUs, warrants the attention of a nationwide surveillance programme to contain the spread, and represents the high incidence of the association of these two carbapenemases in an autochthonous MDR *A. baumannii* indicating that there is a longstanding infection control problem in these hospitals and emergence of MDR GNB harboring genes coding for carbapenemases will undoubtedly limit the use of carbapenems in treating serious infectious in the country and also in the nearby countries.

### ACKNOWLEDGEMENT

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### DISCLOSURE STATEMENT

No competing financial interests exist.

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# INCIDENCE OF VIRAL HEPATITIS AMONG WOMEN ADMITTED TO LABOUR WARD AT MISRATA CENTRAL HOSPITAL

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## ABSTRACT

It is imperative for pregnant women to know their hepatitis status to prevent transmission to their newborn during delivery. Serological screening for viral hepatitis should be offered to pregnant women, as babies born to a mother with hepatitis B have 90% chance of developing chronic hepatitis B if they are not properly treated at birth. So postnatal intervention can be offered to women infected with Hepatitis B to decrease such risk of transmission. To evaluate the incidence of viral hepatitis among women in their third trimester admitted to labour ward, and to determine the magnitude the problem in our locality. A retrospective study was conducted targeting all women delivered in the Department of Obstetrics & Gynaecology at Misrata Central Hospital; patient's data was obtained from medical records of these women during the year 2016. All of them were screened for viral infections (Hepatitis B & C as well as HIV) during their antenatal care in the third trimester. During delivery proper preventive techniques are implemented, including effective 'decontaminate the hands', and use rigorous infection control procedures. For needle stick injuries or splash of body fluid with hepatitis B are recorded and immune prophylaxis is given. Appropriate hygienic precautions should be taken for newborn. In addition to routine vaccination; newborn to HBsAg-positive mothers should receive passive immunization with HBIG at birth. A total of 7142 deliveries including 156 multiple births were recorded. Of them 52 had viral hepatitis B giving an incidence of 0.7%. Hepatitis B represented 91.2% of the cases while hepatitis C accounted for 9.8% of the cases. They were aged between 18 and 42 years. 90% of them were Libyan and 48% were resident outside Misrata. 79% were multiparous. Of the total cases 32.7% were delivered by Cesarean section. The current study revealed a low incidence of Hepatitis B and C in our locality.

**KEY WORDS:** Pregnancy, Hepatitis B, Transmission, Immune prophylaxis.

## INTRODUCTION

Prompt identification of chronic infection with viral Hepatitis B (HBV) enables infected pregnant woman to receive necessary care to prevent or delay onset of liver disease and to receive services to prevent transmission to newborn. While parenteral transmission is still common in children living in developing countries, perinatal transmission is now the leading cause of HCV transmission in developed countries. The absence of an HCV vaccine or approved therapy during pregnancy means that prevention of vertical transmission is still not possible. However, a low vertical transmission rate of 3–5%, a high rate of spontaneous clearance (25–50%) and delayed morbidity have resulted in HCV being overlooked in pregnant women and their infants<sup>(1)</sup>. The risk of vertical transmission of HBV is 70–90% when the woman is hepatitis B e antigen (HBeAg) positive, and around 40% when HBeAg is absent. So Screening for infectious diseases is an integral aspect of antenatal care<sup>(2-5)</sup>. Routine screening is offered to all pregnant women on the basis that early detection and treatment can reduce adverse perinatal outcomes. Intrauterine hepatitis B virus infection has been suggested to be caused by transplacental transmission that cannot be blocked by hepatitis B vaccine. This would decrease the effectiveness of hepatitis B vaccine. The main risk factors for intra-

uterine HBV infection are maternal serum HBeAg positivity, history of threatened preterm labor, and HBV in the placenta especially the villous capillary endothelial cells<sup>(6)</sup>. Maternal infection with either HBV or HCV has been linked to adverse pregnancy and birth outcomes, including mother-to-child transmission (MTCT). HBV infection does not carry a higher risk of birth defects, but seems to be associated with a higher incidence of low birth weight among infants born to mothers with acute infection during pregnancy<sup>(7)</sup>. In a previous study; acute maternal hepatitis (type B or nontype B) had no effect on the incidence of congenital malformations, stillbirths, abortions, or intrauterine malnutrition. However, acute hepatitis did increase the incidence of prematurity<sup>(8)</sup>. Although MTCT for HBV has been reduced to approximately 5% overall in countries those have instituted postpartum neonatal HBV vaccination and immunoprophylaxis with hepatitis B immune globulin<sup>(9)</sup>. Chronic hepatitis B virus infection remains endemic in many parts of the world and there are over 2 billion infected individuals worldwide<sup>(10)</sup>. High prevalence regions for HBV were found in Sub-Saharan Africa, most of Asia and the Pacific Islands<sup>(11)</sup>. Our objective is to evaluate the incidence of viral hepatitis among women in their third trimester admitted to labour ward, and to determine the magnitude the problem in our locality.

## MATERIAL & METHODS

A retrospective study was conducted targeting all women delivered in the Department of Obstetrics & Gynaecology at Misrata Central Hospital; patient's data were obtained from medical records of these women between 1<sup>st</sup> of January to 31<sup>st</sup> of December 2016.

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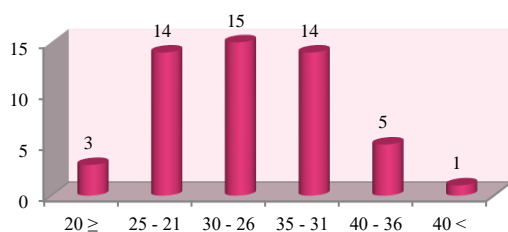


During this period there were 7142 births including 2816 Cesarean births. The data was collected, analyzed and statistical comparison using test of difference of proportions Z-score statistic at 5% of significance where appropriate. As Preventive Services; all pregnant women were screened for viral infections including Hepatitis B & C as well as HIV by ELISA techniques during their antenatal care in the third trimester, regardless of previous testing or vaccination. Liver function tests should also be performed in women who are HBsAg positive as an assessment of liver damage after confirmation by PCR techniques. Also, patient's family screening and immunization will be carried out during antenatal care. Women arriving in labour who have not received antenatal care or have not screened before are to be offered infectious diseases screening after her admission to Labour Ward.

Prevention of perinatal HBV infection begins with good communication regarding maternal HBV status before delivery and Patient's education about the need for infant immune prophylaxis at birth. During delivery proper preventive techniques are implemented, all staff should follow rules on infection prevention by ensuring that they effectively 'decontaminate their hands' before and after each procedure and use rigorous infection control procedures. For needle stick injuries or splash of body fluid to eyes/mouth from patients with hepatitis B are recorded and immune prophylaxis is given. Also, appropriate hygienic precautions should be taken for newborn. In addition to routine vaccination; infants born to HBsAg-positive mothers should receive passive immunization with HBIG at birth (preferably within 12 hours of birth).

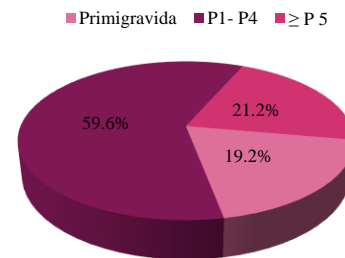
## RESULTS

A total of 7142 deliveries including 156 multiple births were recorded. Of them 52 had viral hepatitis B giving an incidence of 0.74%. Hepatitis B represented 91.2% of the cases while hepatitis C accounted for 9.8% of the cases giving an incidence of 0.07%. All patients with viral hepatitis either B or C were chronic cases and had not received any treatment regarding the viral hepatitis during the course of pregnancy. They were aged between 18 and 42 years, and most common age group was that between 26 and 30 years old represented 28.8% of the total cases of chronic HBV; (figure 1) shows age distribution of patients having HBV. Majority of the cases having HBV (90%) were Libyan and 48% were resident outside Misrata.



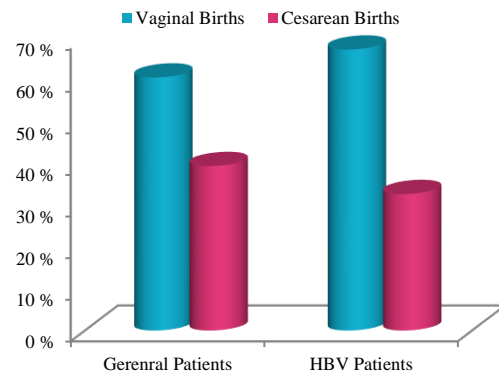
(Figure 1) Distribution of women according to age.

Regarding parity; 19.2% were primigravidae while grandmultiparae represented 21.2% of the total cases of HBV shown in (figure 2).



(Figure 2) Distribution of women according to parity

Of the total cases of HBV; 32.7% were delivered by Cesarean section which is lower as compared to the general Cesarean section rate (39.4%) during the study period however the difference was not significant, (figure 3). There was one maternal death because of postpartum hemorrhage; she had history of recurrent epistaxis and hepatomegaly.



(Figure 3) Mod of delivery

## DISCUSSION

Hepatitis B virus (HBV) infection is a worldwide health problem. The World Health Organization (WHO) estimates that more than 2 billion people have been infected with HBV virus at some point in their lives and 350 million people through the world continue to carry chronic HBV infection, of which almost one million die annually because of HBV-related liver disease<sup>(12)</sup>. Mother-to-child transmission of HBV has been recognized as the major cause of chronic HBV infection, particularly in highly endemic areas such as Southeast Asia and Africa<sup>(13)</sup>. Therefore, prevention of mother-to-child transmission of HBV is the cornerstone to control the infection. Active or passive immunization or both reduces the risk of vertical transmission by 90%<sup>(14)</sup>. Routine antenatal screening for hepatitis B by Elisa techniques for all pregnant women before labour became a policy in our Department of Obstetrics & Gynaecology since 2013. Pregnancy is not a contraindication to vaccination, so pregnant women at risk for HBV infection should receive hepatitis B vaccination<sup>(15,16)</sup>. Also administration of HBIG or antiviral

therapy to HBV carrier mothers during pregnancy is effective in reducing MTCT<sup>(17)</sup>.

Worldwide, the majority of persons with CHB were infected at birth or in early childhood. While the incidence of hepatitis C virus infection among pregnant women included in our study was very low (0.07%) comparing to that reported worldwide between 1 and 8%<sup>(1)</sup>; the incidence of hepatitis B in pregnant women in our study was found to be 0.74% of the total reviewed parturients during the year 2016. The vast majority was known cases of viral Hepatitis B, and few infected women were newly diagnosed in pregnancy and not already under the care of infectology services. Our finding of low incidence is much lower than previously reported in China which was 6.7%<sup>(18)</sup>. Zhang L et al.<sup>(18)</sup> concluded that the passive-active immunization is necessary for neonates of HBeAg-positive mothers. Our finding regarding maternal age revealed that the commonest age group was between 26 and 30 years old, Zhang L et al.<sup>(18)</sup> Mother's age < 28 years and neonate receiving vaccine only were the risk factors for HBV mother-to-infant transmission. Additionally; they have reported that breastfeeding did not put children at risk of our policy regarding mode of delivery of women with HBV positive; if no contraindication for vaginal delivery they can deliver vaginally and no routine for elective Cesarean section, only one-third of studied cases (32.7%) delivered by Cesarean section for different indications. While it has been proposed that elective caesarean section may be a means to reduce mother to child transmission, mode of delivery has not been shown to affect perinatal transmission rates in most studies<sup>(9,20)</sup>. However Pan CQ et al.<sup>(21)</sup> have found a significantly lower rate of vertical transmission of HBV infection to infants delivered by elective Cesarean Section, compared with those delivered vaginally or by urgent Cesarean Section. Furthermore; elective cesarean sections for HBeAg-positive mothers with pre-delivery levels of HBV DNA  $\geq 1,000,000$  copies/mL could reduce vertical transmission. It was concluded that with the recommended immunoprophylaxis against hepatitis B, elective cesarean sections did not reduce the risk of mother-to-child transmission of HBV. Therefore, elective cesarean sections should not be used in HBsAg-positive pregnant women to prevent mother-to-child transmission of HBV<sup>(22)</sup>.

### CONCLUSION

The current study revealed a low incidence of Hepatitis B and C in our locality

### RECOMMENDATIONS

We should enhance identification of HBV infections through screening all pregnant women in their early pregnancy, so can screen the family's members, plan for antiviral medication to women with a high level of virus in the blood to minimize the risk of transmission, and also can refer them for hepatologist.

An educational program should be implemented to increase the patient's awareness of their chronic HBV infection

We should enhance infection control practice, education, and enforcement are critical strategies including vaccination of all health care workers. Also, medical devices should be used correctly for every patient to eliminate mother-to-child transmission of HBV.

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# ANTIBIOTIC MISUSE AMONG PREGNANT WOMEN IN MISURATA CITY

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## ABSTRACT

There is increasing evidence that the self-medications among pregnant women is common in many developing countries. Despite the adverse impact on pregnancy, there are few programs available for their control. The objective of this study was to assess the level of self-medication by antibiotics amongst Libyan pregnant women in order to determine the drug misuse, development of resistance and the possible harmful effects on fetus. Four hundred pregnant women, aged between 18 and 40 years were drawn from outpatient obstetric clinics and hospitals in Misurata city and assessed for self-medication by antibiotics, adapted from a self-report questionnaire based on the WHO guidelines for antibiotic misuse survey. Of the 400 pregnant women assessed, 61 (15.3%) indulged in the self-use of antibiotics for common cold, fever cough and pelvic pains (not prescribed from the antenatal clinic), 107 (26.8%) didn't complete the full course of the drug (prescribed from the antenatal clinic) when they felt any improvement. A total of 211(52.7 %) pregnant women complete the full course of the drug and 21 (5.2%) are not aware in antibiotic misuse in pregnancy. There was a significant difference in the pregnant women whom completed the full course of the drug and those don't complete it. ( $X^2=10.97$ ,  $p=0.001$ ); There was also a significant difference between the presence of current pregnancy illness and antibiotic misuse ( $X^2=9.04$ ,  $p=0.001$ ) There was no significant difference among pregnant women who were highly educated compared to those with little or no education. However, the level of education has no strong impact in the self-usage of antibiotics as shown above. No fetal complications related to misuse was detected. This study shows that self-medication is not uncommon among pregnant women in our environment. There is need for adequate education of pregnant women during antenatal clinics on the potential danger of antibiotic self-medication so as to prevent child and maternal morbidity and mortality.

**KEY WORDS:** Antibiotic, Self-Medication, Pregnancy, fetal, Side effects.

## INTRODUCTION

Antimicrobial resistance is one of the greatest threats to global population health in this century and it is a major contributor to rising healthcare costs worldwide. The primary cause of this resistance is antibiotic misuse, especially routine inappropriate use of antibiotics for self-limiting illnesses. Over prescription of antibiotics is pervasive leading to very high and increasing rates of antimicrobial resistance in both hospital-acquired and community-acquired infection. Self-medication is identified by the WHO as a major leading factor to antibiotic misuse & overuse and resistance, Self-medication is a considerable problem all over the world in face of current global economic downturn<sup>(1,2)</sup>.

A large number of countries are facing serious health challenges with people finding it difficult to meet their health needs, In developed countries, self-medication is not uncommon, but the practice is guided because people are enlightened and could derive adequate information from various sources<sup>(3)</sup>. Consequently, it is often regarded as consumers' luxury and very attractive. Evidence suggests that many people involved in self-medication tend to acquire knowledge of the practice from relatives, neighbors, medicine dealers, and sometimes media<sup>(4)</sup>. The situation in developing countries is frightening, where there is poor medical services and lack of professional control of pharmaceutical products<sup>(1)</sup>.

This therefore forces people to self-medicate themselves with various forms of medicines for different medical complaints<sup>(5,6)</sup>, Even though the practice is high, there is scarcity of data on the impact on the people, A few of the studies that had been carried out have shown potential harmful effects on both the fetus and mothers usually exposed to un-prescribed antibiotics<sup>(7,8,9)</sup>.

Several factors including poverty, cultural perception of certain diseases' entity and their perceived responses to indigenous medications have been widely reported as indicators in developing countries making the practice a necessity, This makes it more dangerous as basic knowledge concerning the pharmacological properties of these drugs may be lacking<sup>(10)</sup>.

## MATERIAL AND METHODS

The aim of our study was to explore the knowledge and behaviors of pregnant ladies in relation to antibiotic use and misuse in Misurata city.

This study was carried out at Misurata city, it has a population of about ½ million people. Data collection using a one-stage random sampling method, 400 pregnant women were randomly selected and screened for self-medication by antibiotics, using a questionnaire adapted from of self-report based on the World Health Organization guidelines for antibiotics misuse<sup>(15)</sup>. Commonly available antibiotics were added to the list of substances in the questionnaire. Participants were also asked to indicate the reasons for using the medicines.

Information on age, marital status, educational level and occupation were elicited through a semi-structured socio-demographic questionnaire. Data

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analysis: The results of the study were analyzed using Statistical Package for Social Sciences (SPSS 17.0). The proportion of the pregnant women involved in the use of different antibiotics was found from the respondents. Sample means and percentages were calculated from which simple frequency tables were created. Standard deviation from the mean was calculated and comparisons of categorical data were done using Chi-square. The P-value of less than or equal to 0.05 was used to determine the statistical significance.

## RESULTS

(Table 1) Age distribution

Age group	Frequency	Percent
<20	133	33.3 %
20 - <30	130	32.5 %
30 - <40	97	24.2 %
> 40	40	10.0 %
Total	400	100.0 %

(Table 2) Nationality distribution

Nationality	Frequency	Percent
Libyan	332	83.0 %
Non-Libyan	68	17.0 %
Total	400	100.0

(Table 3) Parity distribution

Parity	Frequency	Percent
PG	85	21.3 %
P1 – P3	149	37.3 %
P4 or more	166	41.5 %
Total	400	100.0

(Table 4) Occupation distribution

Occupation	Frequency	Percent
Employed	244	61.0 %
Non-Employed	156	39.0 %
Total	400	100.0

(Table 5) Distribution of Level of education

Education	Frequency	Percent
High	61	15.3 %
Moderate	135	33.8 %
Low	204	51.0 %
Total	400	100.0

(Table 6) Distribution according to gestational age

Gest- Age	Frequency	Percent
<20 weeks	222	55.5 %
>20 weeks	178	44.5 %
Total	400	100.0

(Table 7) Distribution according antibiotic course completion

Completion	Frequency	Percent
Completed	272	68.0 %
In-completed	128	32.0 %
Total	400	100.0

(Table 8) Distribution according to antibiotics side-effects

side-effects	Frequency	Percent
No	264	66.0 %
Gastric upset	76	19.0 %
Diarrhea	60	15.0 %
Total	400	100.0

(Table 9) Distribution according awareness to antibiotics misuse

Awareness	Frequency	Percent
Yes	379	94.8 %
No	21	5.2 %
Total	400	100.0

(Table 10) Distribution according to antibiotics use & misuse

	Awareness	Frequency	Percent
Self	medication	61	15.3 %
	Incomplete course	107	26.8 %
	Completed course	211	52.7 %
	Un-aware	21	5.2 %
	Total	400	100

Out of the 400-pregnant women participants, 61 (15.3%) indulged in the self-use of antibiotics for common cold, fever cough and pelvic pains (not prescribed from the antenatal clinic), 107(26.8%) didn't complete the full course of the drug (prescribed from the antenatal clinic) when they felt any improvement. A total of 211 (52.7 %) pregnant women complete the full course of the drug and 21 (5.2%) were not aware in antibiotic misuse in pregnancy while the rest were aware of it 379 (94.8%). There was a significant difference in the pregnant women whom completed the full course of the drug and those don't complete it. ( $X^2=10.97$ ,  $p=0.001$ ); There was also a significant difference between the presence of current pregnancy illness and antibiotic misuse ( $X^2=9.04$ ,  $p=0.001$ ).

There was no significant difference among pregnant women who were highly educated compared to those with little or no education. However, the level of education had no strong impact in the self-usage of antibiotics as shown above. No fetal complications related to misuse was detected.

Tables from 1-10 show the socio-demographic characteristics of pregnant women. Of the 400-pregnant women participants, 133 (33.3%) were aged below 20 years, 130 (32.2%) between 20 and 29 years, 97 (24.2%) 30-39 years, while 40 (10 %) were aged above 40 years. Most of the cases 83 % were Libyan, more than 70% of the cases were parous ladies, 61% are employed, most of them were school teachers while the others were employees, medical staff were excluded to avoid any bias in the study.

A total of 61 (15,3%) are highly educated (Ph.D. & master degree or a high diploma). 135 (33.7%) had a moderate education (graduates of University) while 204 (51%) are either graduated from institutes or primary & secondary schools.

Around 222 (55%) of the cases were in their first half of pregnancy, 61 (66.7%) were had a self-medication with antibiotic while the rest 107 (26.8%) hadn't complete the full course of antibiotics, 211 (52.7) had completed their full course. No major side effects were observed in most of cases apart of gastric upset.

### DISCUSSION

The findings from this study suggest that the practice of self-medication is not uncommon among pregnant women in our environment. In this study, various types of antibiotics were used but the commonly used are ampicillins and cephalosporins which are somehow considered safe in pregnancy in regard to fetal effects.

These findings were frightening and very serious in view of the poor knowledge of the safety profiles of these drugs among the pregnant women, the difficulty in estimating the right and adequate dosages may pose serious problem<sup>(1)</sup>. One major concern would be the potential harmful effects on the fetus, Therefore, the misuse of antibiotics for whatsoever reason during pregnancy should be discouraged.

This highlights the need for the effective programs that would target on prevention of self-medication among pregnant women and intervention to arrest complications like antibiotic resistance<sup>(11,12,13)</sup>.

The act of self-medication could be dangerous considering the possible poor knowledge of Pharmacodynamics of these medicines<sup>(4)</sup>.

This is more worrisome as the sources and instructions concerning the use of these medicines are not from professionals. One major concern would be issue of inadequate dosing and development of antibiotic resistance, The widespread use of suboptimal dosages of antibiotics is a major threat, contributing to the development of antibiotics resistant bacterial strains<sup>(14,15,16)</sup>.

Apart from the resistance that may result from inadequate dosing, exposure to some antibiotics (such as septrin, choramphenicol and tetracycline which are dangerous and contraindicated during pregnancy) was in the early month of pregnancy, these children could suffer from serious complications including, grey baby syndrome, deafness, jaundice, brain damage, anemia and impaired bone development and respiratory distress<sup>(17)</sup>.

Perhaps the most clinically relevant aspect of the pregnancy microbiome is antibiotic treatment during pregnancy. Antibiotics account for 80 % of all prescribed medication in pregnancy yet surprisingly, few published human studies have carefully evaluated the direct effects of antibiotics during pregnancy on either the maternal or fetal microbiome, or evaluated long-term sequel of such antibiotic use. Thus, there may be a reason for caution in prescribing antibiotics during pregnancy<sup>(18)</sup>.

There is a vast amount of data showing that antibiotic use is not free of serious adverse events. Among other complications, antibiotics have been associated with allergic reactions, gastrointestinal disturbances, cardiac arrhythmia, and death<sup>(19)</sup>. A major problem associated with antibiotic overuse is the development of multi-resistant bacteria, the necessity to treat most cases of symptomatic bacterial infections is clear, but it is also clear that during the last 20 years there has been an inappropriate overuse of antibiotics to treat respiratory, ear, nose and throat, urinary, or genital infections. It can be stated also that antibiotics have been and still are overused in obstetrics<sup>(20,21)</sup>.

It has been suggested that exposure to antibiotics during fetal/neonatal life affects the development of allergic diseases via their adverse and possible long-term effect on gut microbes of both the mother and child and vaginal microbes of the mother. Antibiotic use may delay and interfere with the early colonization of the child's gut microbes<sup>(22)</sup>. In turn, this delay or aberrant colonization may interfere with the development and maturation of the child's immune system, and thus play a role in the development of allergy and disease<sup>(23,24,25)</sup>. It has been reported recently that antibiotic use early in life is associated with the risk of childhood asthma, allergy, atopic dermatitis, eosinophilic Oesophagitis, neonatal Candidiasis, and celiac disease<sup>(26,27,28,29,30,31,32,33,34,35)</sup>.

Antibiotic overuse during pregnancy is associated with the emergence of many antibiotic-resistant organisms. Rates of GBS resistant to erythromycin (one of the antibiotics of choice after preterm PROM and given in most UK hospitals for that indication) is as high as 35%<sup>(36,37)</sup>. Their use during pregnancy had been shown also to be associated with the selection of resistant strains of *Escherichia coli*, which has been reported increasingly in neonatal sepsis, especially in very preterm infants<sup>(20,38,39)</sup>.

Some of these risks are inherent to the existing policies for the prevention of pregnancy complications. The policy for the routine administration of antibiotics during cesarean section could be questioned as the risk for postoperative maternal infection varies widely. Among low-risk women (elective cesarean section with intact membranes), approximately 1000 women should receive antibiotics in order to prevent 6 cases of Endometritis and 4.4 cases of abdominal wound infections<sup>(40,41,42)</sup>.

In women at higher risk (emergency cesarean section, ruptured membranes, obesity, etc.), the number needed to treat is much lower (between 5–25 to avoid one case of maternal postpartum morbidity), but still varies substantially depending on the country<sup>(43)</sup>. Without further research on long-term infant outcomes, the safest strategy is still to administer antibiotic prophylaxis after fetal cord clamping.

Our study shown the variation in the practice and use of self-medication among the participants. In this study, about 15% of the pregnant women with

high level of education used antibiotics the same way as 33% and 51 with moderately and low education respectively. This, when compared with 51% of them with low level of education involved in the use of the antibiotics, is very significant as the less education the more the misuse. These findings seem to suggest that the level of education influences the type and nature of the antibiotic used. Although earlier studies have association self-medication with factors such as self-employment, unemployment and third trimester of pregnancy, However, certain reasons may be responsible for this differential practice in this study. like, the use of certain new generations of antibiotics like cephalosporins by women with high level of education and the use of traditional ones like ampicillins by those with low level of education may be due to health beliefs, which to a large extent, determines the emotional and behavioral responses to illness<sup>(18)</sup>. These beliefs involve expectancy and perceived benefits, as well as outcome for initiating and maintaining treatment. There is abundance of evidence that this perception plays a significant role in health-seeking behavior and pathway to care<sup>(10)</sup>.

Although various reasons have been proffered for this practice, the implications could be overwhelming, as issues related to possible drug interactions could be a major problem to both mother and the unborn child<sup>(13)</sup>. Therefore, health care providers must be aware of this trend, in order to weigh the therapeutic benefits to mothers and the potential risk to the developing fetus<sup>(9)</sup>. It is essential to routinely inquire about the woman's self-medication practice so as to provide appropriate advice during antenatal care.

#### CONCLUSION

The findings of this study have shown that the practice of self-medication with antibiotics among pregnant women is on the increase in our environment. Therefore, there is need to reduce this practice by improving the quality of antenatal care services to include adequate health education on major issues capable of influencing the health behaviors of our pregnant women. In order to achieve the development goal aimed at reducing child and maternal morbidity and mortality, there is need to upgrade healthcare facilities and embark on regular massive enlightenment campaigns, especially in rural areas to encourage increased healthcare services utilization. Since many of these pregnant women for reasons known to them and irrespective of their level of education practicing the self-medication, there is need for education of the public on the danger of self-medication and the potential harmful effect on the unborn child. This will ensure attitudinal change and encourage safer practice. More importantly, there is a pressing need for strict drug prescription control. Efforts must also be made to make sure that medicines are only available to individuals on prescription from a physician following a consultation, and

monitoring to ensure that drugs are not prescribed and sold indiscriminately by those without basic pharmacological knowledge.

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# OCCLUSAL TRAITS AND ORTHODONTIC TREATMENT NEED AMONG SCHOOLCHILDREN IN MISURATA, LIBYA

RNLNL  
139/2018

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## ABSTRACT

The aim of this study is to determine the orthodontic treatment need of Libyan schoolchildren using the Index of Orthodontic Treatment Need (IOTN) and the amount of contribution of the various occlusal traits in rating of the children in IOTN scale. This data provides solid information for planning of orthodontic care and give hard base about the most occlusal traits in producing malocclusion in Libyan schoolchildren. In this cross-sectional study of School-going children aged 14-15 years, their parents consented to an intra-oral examination. A total of seventeen schools in Misurata were selected and a total of 1,050 children were entered into the study. Five children (0.5%) had previously received orthodontic treatment and these children were excluded from the study. The gender of the sample population was remarkably even and comprised 522 males (49.7%) and 528 females (50.3%). The mean age of male students was 15.3 years old, while that of female students 15.5 years old. However, the age range was slightly larger with the youngest students 13 and 17 years old. The study found that 27 percent of children were in Dental Health Component grade 5 and 4 and therefore in definite need for orthodontic treatment. Less than 24 percent of children (grade 3) were in borderline need and 49% (grade 2 and 1) had little or no need for treatment. The contribution of the main occlusal features responsible for definite need respectively as follow; severe contact displacement (4d) 34.3 per cent, overjet greater than 6 mm but less than or equal 9 mm (4a) 17.3 per cent, crossbite with displacement greater than 2 mm (4c) 14.1 per cent, impeded eruption (5i) 14.1 per cent, less extensive hypodontia (4h) 7.1 per cent, overjet greater than 9 mm (5a) 6 per cent, partially erupted, tipped and impacted against adjacent teeth (4t) 4.6 per cent, submerged deciduous teeth (5s) 1.1 per cent, reverse overjet greater than 3.5 mm with reported masticatory and speech difficulties (5m) 0.7 per cent and 0.4 percent for both extreme lateral or anterior open bite greater than 4 mm (4e) and presence of supernumerary teeth (4x).

**KEYWORDS:** Schoolchildren, IOTN, Dental Health Component (DHC), Malocclusion and Occlusal Traits.

## INTRODUCTION

Malocclusions prevalence is second only to tooth decay and periodontal disease, and therefore rank third among all worldwide public health dental disease priorities [World Health Organization, 1997]. Therefore, rational planning of an orthodontic treatment service on a population basis is essential and requires baseline data on the prevalence of different types of malocclusion.

In clinical orthodontics, malocclusion assessment remains problematic because there are no generally accepted criteria to define normal or abnormal occlusal status to date.

One of the previous Libyan studies was investigated by Gardiner (1982) surveyed the prevalence of malocclusion in 479 schoolchildren in Benghazi, Libya. Only Angle's classification was used to assess the antero-posterior dental arch relationship and Crowding was detected in 31 percent of the sample. Another Libyan study was done by Ommar (1994), the occlusal status of 2015 Libyan schoolchildren, aged between 7 – 16 years, who found that 94.5% had Angle's Class I molar relationship. Ninety two percent had an overjet between 2 and 5mm, 4.1% had an overjet of 6mm or greater while 3.8% had an overjet of 1mm or less. Only 13.4% of the sample had crowding.

The other study was done by Iman Bugaighis and Divakar Karanth in 2013 they found that the prevalence of malocclusion in the Benghazi population of Libya is among the highest reported in

the literature. These studies are of limited value since they did not use a scientifically validated index of orthodontic treatment need.

The aim of the present survey was to document the prevalence of individual traits of malocclusion in Libyan children aged 14 to 15 years old, and the contribution of these traits in determining the priority of needing for orthodontic treatment by using IOTN index.

The index of orthodontic treatment need (IOTN) developed by Brook and Shaw (1989) is gaining wide acceptance in the UK and other parts of Europe. It has approved to be a valid, reliable and quick index to assess orthodontic treatment need (Burden and Holmes, 1994).

The IOTN is composed of two independent components. One component records the dental health need for orthodontic treatment. The other component records the aesthetic need for orthodontic treatment. In its original form, the Dental Health Component comprises a 5-grade index and the Aesthetic Component consists of a 10-grade standardized ranking scale of dental attractiveness.

The IOTN attempts to rank malocclusion in terms of the impact of various occlusal traits on an individual's dental health or perceived aesthetic impairment. Social research strongly indicates that aesthetic impairment is associated with psycho-social disadvantage.

IOTN is also quick and reliable when used to assess treatment need at a community level, as it only

records the most severe occlusal traits (Burden and Holmes).

In this article only DHC is used and its relation with the most common occlusal traits which are affecting directly to rate the subject on IOTN.

### MATERIALS AND METHODS

The present study was carried out in school-going children aged 14 – 15 years. The children parents were consented to an intra-oral examination. A total of seventeen schools from different parts of Misurata were selected and a total of 1,050 children were entered into the study. Five children (0.5%) had previously received orthodontic treatment and these children were excluded from the study.

Prior to carrying out the study the investigator was calibrated in the use of both components of the Index of Orthodontic Treatment Need.

A data collection sheet (Appendix) listing all clinical data to be collected was prepared beforehand. At examination the subject was seated and under artificial lighting conditions an intra-oral examination was carried out.

Clinical examination was completed within the school with the subject seated and using a portable dental light, a dental mirror and an orthodontic stainless-steel ruler.

Each student's occlusion was assessed using IOTN. The DHC of the IOTN records the various occlusal traits into five grades according to the severity and the need for orthodontic treatment. Grades 1 and 2 represent no need for treatment, grade 3 borderline, and grades 4 and 5 are considered to be a definite need for orthodontic treatment.

To record the dental health component, score a disposable IOTN ruler was used.

The overjet was measured by selecting the largest overjet measurement of the four upper incisors. The relationship of the upper to lower centerline was recorded in relation to midfacial plane. The result was recorded on a reference grid. The overbite recording zone was all four incisors and overbite recorded as either normal, increased or decreased. The overbite was classified as complete or incomplete or traumatic. Anterior posterior or lateral open bites were recorded and the maximum opening recorded in millimeters. The molar relationship on the left and right sides according to Angle's classification was recorded as was the canine relationship. A visual assessment of crowding was made in both arches and the largest contact point displacement, measured in millimeters.

3.e Lateral or anterior open bite greater than 2 mm but less than or equal to 4 mm.

3.f Deep overbite complete on gingival or palatal tissues but no trauma.

limeters, was made in each arch. Anterior and or posterior crossbite, if present and the magnitude of any associated displacement was recorded.

### The original dental health component of IOTN: Grade 5 (Need treatment)

5.h Extensive hypodontia with restorative implications (more than 1 tooth missing in any quadrant) requiring pre-restorative orthodontics.

5.i Impeded eruption of teeth (except for third molars) due to crowding, displacement, the presence of supernumerary teeth, retained deciduous teeth and any pathological cause.

5.a Increased overjet greater than 9 mm.

5.m Reverse overjet greater than 3.5 mm with reported masticatory and speech difficulties.

5.p Defects of cleft lip and palate and other craniofacial anomalies.

5.s Submerged deciduous teeth.

### Grade 4 (Need treatment)

4.h Less extensive hypodontia requiring pre-restorative orthodontics or orthodontic space closure to obviate the need for a prosthesis.

4.a Increased overjet greater than 6 mm but less than or equal to 9 mm.

4.b Reverse overjet greater than 3.5 mm with no masticatory or speech difficulties.

4.m Reverse overjet greater than 1 mm but less than 3.5 mm with recorded masticatory and speech difficulties.

4.c Anterior or posterior crossbites with greater than 2 mm discrepancy between retruded contact position and intercuspal position.

4.l Posterior lingual crossbite with no functional occlusal contact in one or both buccal segments.

4.d Severe contact point displacements greater than 4 mm.

4.e Extreme lateral or anterior open bites greater than 4 mm.

4.f Increased and complete overbite with gingival or palatal trauma.

4.t Partially erupted teeth, tipped and impacted against adjacent teeth.

4.x Presence of supernumerary teeth.

### Grade 3 (Borderline need)

3.a Increased overjet greater than 3.5 mm but less than or equal to 6 mm with incompetent lips.

3.b Reverse overjet greater than 1 mm but less than or equal to 3.5 mm.

3.c Anterior or posterior crossbites with greater than 1 mm but less than or equal to 2 mm discrepancy between retruded contact position and intercuspal position.

3.d Contact point displacements greater than 2 mm but less than or equal to 4 mm.

### Grade 2 (Little need)

2.a Increased overjet greater than 3.5 mm but less than or equal to 6 mm with competent lips.

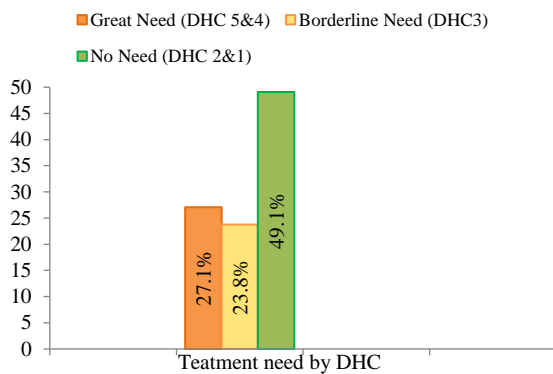
- 2.b Reverse overjet greater than 0 mm but less than or equal to 1 mm.
- 2.c Anterior or posterior crossbite with less than or equal to 1 mm discrepancy between retruded contact position and intercuspal position.
- 2.d Contact point displacements greater than 1 mm but less than or equal to 2 mm.
- 2.e Anterior or posterior open bite greater than 1 mm but less than or equal to 2 mm.
- 2.f Increased overbite greater than or equal 3.5 mm without gingival contact.
- 2.g Prenormal or postnormal occlusions with no other anomalies includes up to half a unit discrepancy.

Grade 1 (No Need)

- 1. Extremely minor malocclusions including contact point displacements less than 1 mm.

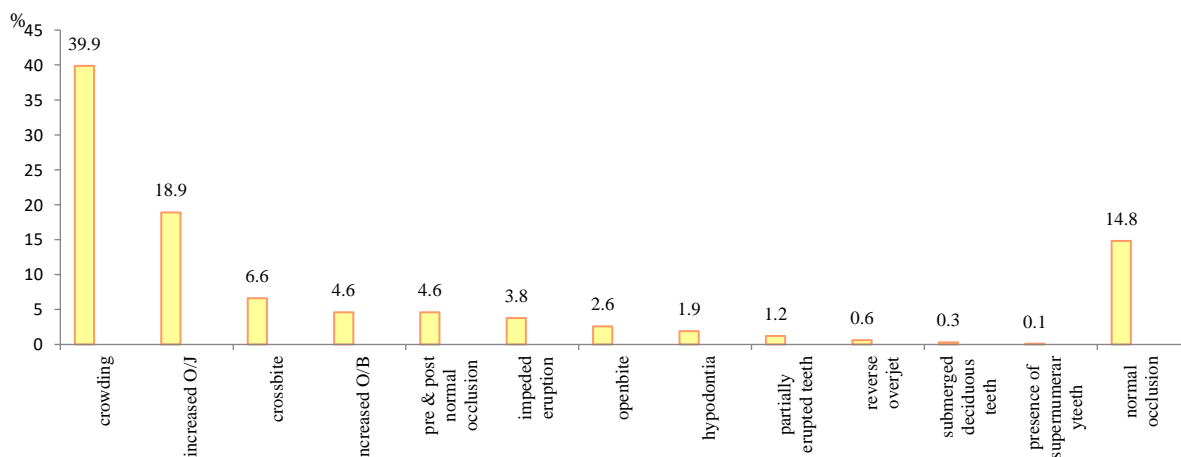
**RESULTS**

The distribution ratings for the DHC of IOTN in the Libyan school population were found as follows; 27.1 per cent in definite need for treatment, 23.8 per cent in borderline need for treatment and 49.1 per cent little or no need for treatment (figure 1).

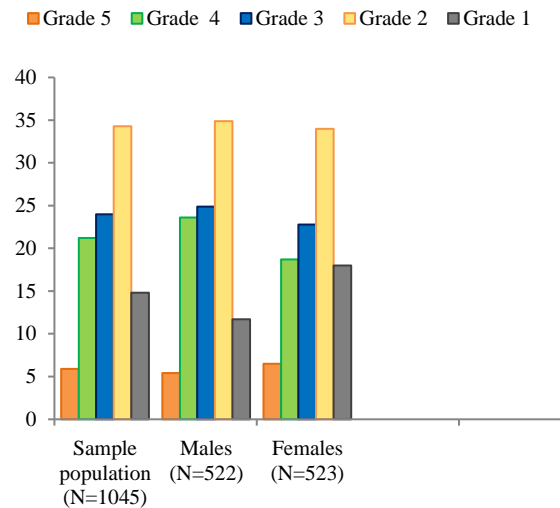


(Figure 1) Distribution of DHC treatment need categories.

(Figure 2) shows the percentage distribution of treatment need among the sample according to the five dental health components grades of the IOTN. The largest proportion of subjects (34.3%) felt into grade two followed by grade three (24%), grade four (21.2%), grade one (14.8%) and grade five (5.9%).

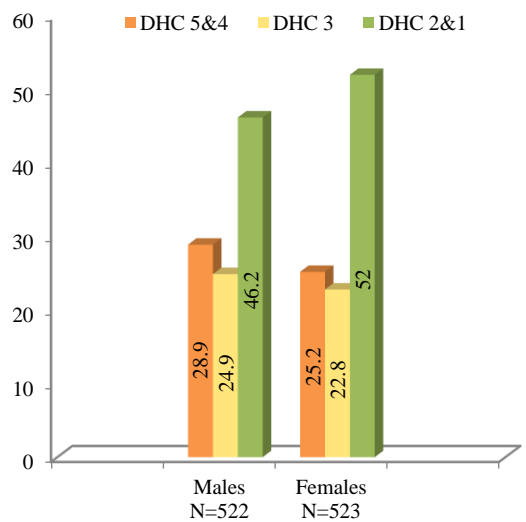


(Figure 4) Distribution of traits within the DHC scores.



(Figure 2) The distribution percentage of the sample according to DHC categories of IOTN.

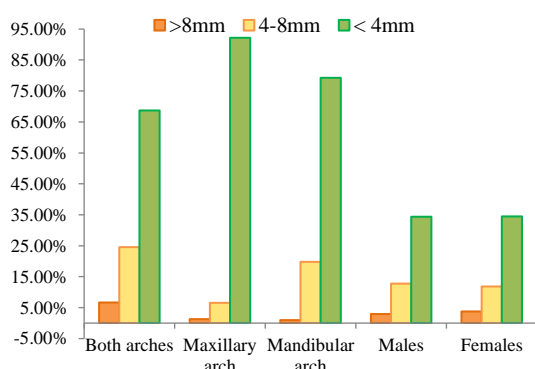
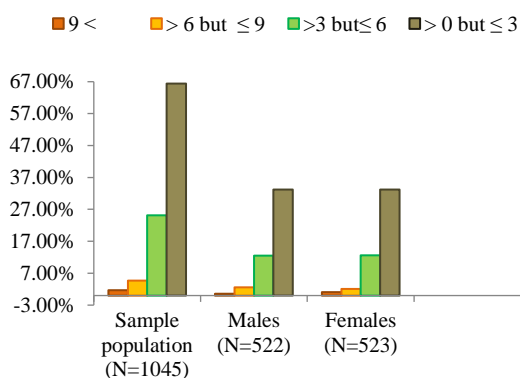
(Figure 3) shows the distribution of DHC treatment need categories according to the gender.



(Figure 3) Distribution of DHC treatment need categories according to the gender.

**(Table 1)** Percentage distribution of DHC scores for subjects with a definite need for treatment (DHC 4 and 5).

IOTN component	Percentage Distribution
4.d	34.3
4.a	17.3
4.c	14.1
5.i	14.1
4.h	7.1
5.a	6.0
4.t	4.6
5.s	1.1
5.m	0.7
4.e	0.4
4.x	0.4
<b>TOTAL</b>	<b>100</b>

**(Figure 5)** Distribution of estimated crowding.**(Figure 6)** Distribution of overjet for all subjects.

### DISCUSSION

The use of an occlusal index to determine treatment need is an objective and effective way to screen patients for orthodontic treatment (Otuyemi OD, Jones SP. and Shaw WC, Richmond S, O'Brien KD, Brook P). It is a safeguard against over-treatment and a meaningful basis for an objective discussion between children, parents, referring general dental practitioners and orthodontists about the need for treatment.

The schoolchildren in the present study are older than those examined in previous studies (Brook and Shaw, 1989; Burden and Holmes, 1994; Richmond et al., 1994). The choice of age was to avoid recording unerupted teeth as impacted teeth or as missing teeth where the teeth in question will erupt uneventfully and also the unexpected residual growth which could affect in the rating of the child in DHC.

Orthodontic treatment need as determined by IOTN has not been previously investigated in Libya. However, the previous studies relied upon subjective criteria to determine whether the subject was within the normal range of occlusal traits or was in need of orthodontic treatment.

IOTN has now gained wide acceptance and is increasingly used to ascertain treatment need in populations. The validity of the use of IOTN index has been verified by several researchers (Richmond et al., 1993; Burden et al., 1994; Burden and Holmes, 1994; Shaw et al., 1995).

The current study revealed the most occlusal traits the children have to rate them in IOTN and then their responsibility in putting children in need of orthodontic treatment.

This will provide baseline data on treatment need in the Libyan 13 – 17-years-old school-going population and allows comparison to be made with studies in other countries for a similar population group. Additionally, the information may be used to plan orthodontic manpower and in the planning of future service provision in the Libyan school-going population. The main occlusal features responsible for allocating children in great need of orthodontic treatment were severe contact displacement (4d) 34.3 per cent, overjet greater than 6 mm but less than or equal 9 mm (4a) 17.3 per cent, crossbite with displacement greater than 2 mm (4c) 14.1 per cent, impeded eruption (5i) 14.1 per cent, less extensive hypodontia (4h) 7.1 per cent, overjet greater than 9 mm (5a) 6 per cent, partially erupted, tipped and impacted against adjacent teeth (4t) 4.6 per cent, submerged deciduous teeth (5s) 1.1 per cent, reverse overjet greater than 3.5 mm with reported masticatory and speech difficulties (5m) 0.7 per cent and 0.4 percent for both extreme lateral or anterior open bite greater than 4 mm (4e) and presence of supernumerary teeth (4x) (figure 4) and (table 1).

### CONCLUSION

The most common occlusal traits respectively found; contact point displacement, increased overjet, crossbite with displacement, impeded eruption and less extensive hypodontia.

Orthodontic treatment need in Libyan children, as determined by DHC, is less than that reported in other populations.

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# A STUDY OF CROSS INFECTION CONTROL WITHIN DENTAL LABORATORIES IN MISURATA CITY

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139/2018

## ABSTRACT

Infection control is an essential part of dentistry. Potential pathogens can be transported to laboratory via orally soiled impressions and dental prostheses. The purpose of this study was to investigate the attitude and behavior of dental laboratories within Misurata city to cross infection control. A survey was conducted to collect data on the cross-infection procedures adopted by 40 dental laboratories within Misurata city from November 2010 to February 2011. The resultant responses to each question of the five-part questionnaire were then analyzed using (SPSS 15.0 for windows). The results of the study demonstrated that the attitudes and procedures of dental laboratories within Misurata city to infection control appeared to be very variable 20% of dental laboratories have documented procedure for infection control. Only 3% of dental laboratories used liquid disinfectants, 15% change their pumice daily and 35% have exhaust systems containing filter. In addition, most of the laboratories (77.5%) devoid running water or separate hand washing facilities. Furthermore, 10% of dental technicians attended infection control training courses and 32.5% believed in immunization against hepatitis B. Moreover, most of dental technicians (55%) were not care about the use of protective measures, during either handling new items or polishing of prostheses. There seems to be a definitive need to provide and distribute formal and obligatory standard of current infection control guidelines and manuals to the dental laboratories.

**KEY WORDS:** Cross infection control, dental laboratories.

## INTRODUCTION

The dental healthcare professionals, including laboratory personnel, are at risk of infection transmission. Therefore, as members of the healthcare profession, it is advised that we ensure a safe working environment to prevent the risk of transmission of blood-borne infection during various stages of dental treatment. To ensure that, the maximum infection control protocols and procedures are being applied in dental operatory<sup>(1,2)</sup>. In these days, dental laboratories widely spread; increased; and they worked in a random way, and there is no care fullness with health in these laboratories that makes the infection spreads throughout these areas<sup>(3)</sup>.

As we specialized in this field we have noticed a lot of errors from dental technicians in their laboratories especially when they did not take protective precautions to prevent transmission of infection. In addition to that, we also noticed that most of dental technicians ignored the control of communicable diseases which transfer through blood from polluted impressions which move toward the laboratory from clinic without any sterilization and preventive measures in place<sup>(4)</sup>.

Most of dental laboratories don't have any programs to control or prevent infection may be due to losing the technical consciousness or the absentness of qualified technicians. Furthermore, the dental technicians are at risk by this infection while they are contributing in treating of patients. The fabrication of prosthesis for infectious disease carriers presents a cross contamination hazard. Dentures, crowns, bridges, impressions, casts and other saliva or blood coated items are all exposed to contamination in the patient's

mouth. Such items can spread infectious agents to similar items within the laboratory, where technicians and other patients are vulnerable to exposure<sup>(5,6)</sup>.

In view of the risk of infection of dental healthcare workers and patients, interruption of possible chains of infection, applied infectious control programs should be in demanded.

Media publicity has increased public awareness of the need for adequate and obvious cross infection control within the dental surgery. Patients now expect and demand high standards of care even though their knowledge of aspects of cross infection control is limited.

As members of the healthcare profession it is advised that we ensure a safe working environment to prevent the risk of transmission of blood-borne viruses and other infectious agents not only from patient to dentist but also from dentist to patient. To ensure a maximum infection control procedure, many of protocols were drawn up to meet standardize universal precaution. However, people always looking for more protective and efficient precautions<sup>(7,8,9)</sup>. The purpose of this study was to investigate the attitude and behavior of dental laboratories to cross infection control within Misurata city.

## MATERIALS AND METHODS

A survey was conducted within Misurata city from November 2010 to February 2011 to collect data on the cross-infection procedures using questionnaire adopted by 40 dental laboratories.

Questionnaire consists of 5 sections:

- The first section collected information on the management of the laboratories. Questions regarding

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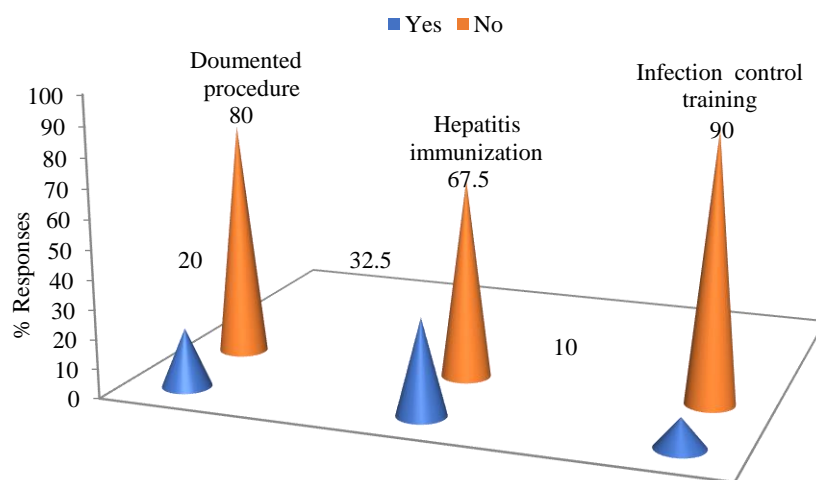
immunization and infection control training of staff were also added in this section.

- The second section sought information on the facilities in the laboratory.
- The third section was related to sterilization and disinfection of laboratory equipment and material like studying casts, impressions and hand pieces.
- The fourth and fifth sections were recorded information about the general environment and the use of personnel protective facilities.

Data was transferred to computer. All questionnaires were entered into the program and statistical analyses were performed by Data Statistical Consultation Services using Statistical Program for Social Science (SPSS 15.0 for windows). Simple frequencies were calculated for all variables.

## RESULTS

The results were reported in the following graphics: as shown in (figure 1), showed the section of the questionnaire related to management procedures of the dental laboratories. It seemed that only 20% of the dental technicians had a documented procedure for infection control, 32.5% believed that dental technicians should immunized against hepatitis B. Considering the topicality of the subject material and the need for adequate infection control training and knowledge, the response rate of 90% that had not attended any infection control training was disappointing high. However, this response rate should not necessarily be interpreted as lack of interest in cross infection control training but may reflect the lack of availability of enough infection control training programs.

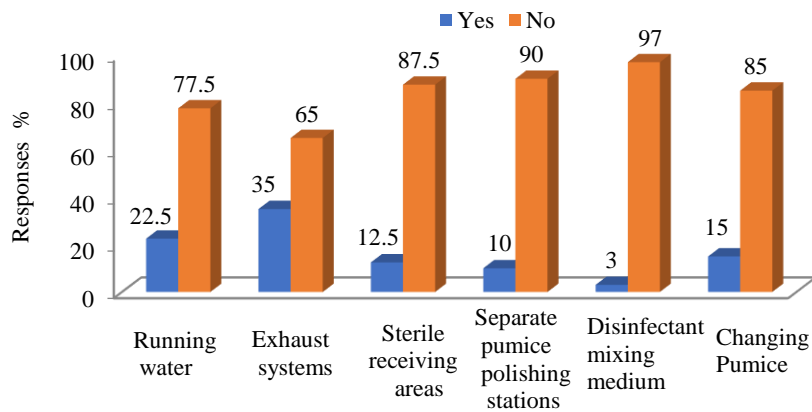


(Figure 1) The management procedures of the dental laboratories.

As shown in (figure 2) a series of questions aimed to find out the facilities available in Dental laboratories within Misurata city. Almost all of the responding laboratories 77.5% had no running water and separate hand washing facilities with a low proportion of 35% having exhaust systems containing filters incorporated in their laboratories. However, this meant that there were still a large proportion of dental laboratories in Misurata city working in a harmful and contaminated environment. For the question number three that inquired if the laboratories had a sterile designated receiving production and shipping areas, with almost an 87.5% of the respondents replying that they did not

had. This meant that a risk of cross infection could occur between contaminated returned work and sterile outgoing work. However, of those responding 90% did not have separate pumice polishing stations for repairing old denture work. This may suggest that many of the dental technicians were not aware of the fact that microorganisms could be present on and below the surface of the denture work and so could be transferred to grinding/polishing equipment and also pumice waiting to be transferred again to the next denture. Moreover, 97% of the dental technicians did not use liquid disinfectant, as a mixing medium in pumice and 85% did not change their pumice daily.

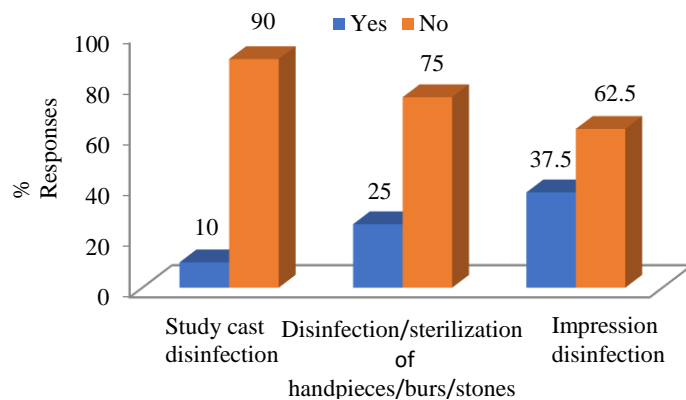




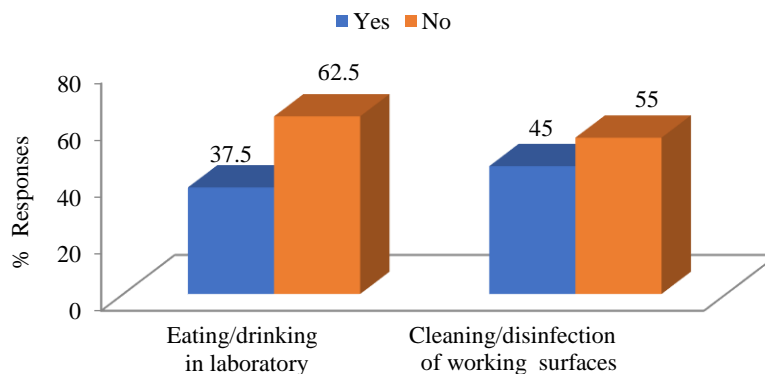
(Figure 2) The facilities available in dental laboratories.

As shown in (figure 3). This part of the questionnaire demonstrated that 90% of the respondents did not routinely disinfect study casts after receiving them from the clinic. With regards to disinfection and sterilization, headpieces, burs and stone equipment appeared to be the least popular whereas, greater efforts were made by the respondents in the routine infection control of impressions. Due to the fact that many of dental technicians require impressions to be previously disinfected at the dental practice, 62.5% of the respondents did not disinfect impressions upon arrival at the laboratory. In relation to laboratory hygiene, 37.5% of the laboratory personnel eat or drink in the dental laboratory, whereas, eating,

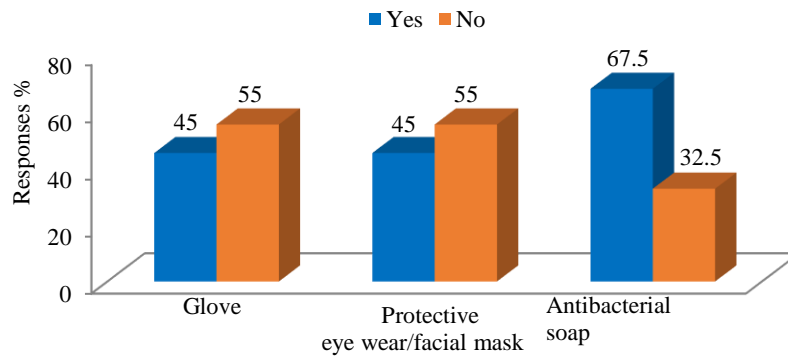
drinking or smoking should be prohibited in the laboratory work area. 45% of dental technicians have cleaning and disinfecting of the work surface areas as shown in (figure 4). Regarding personnel protective equipment, glove wearing when handling new items received in the laboratory was practiced by 45% of the respondents. It is known that 45% of the dental technicians surveyed wore protective eyewear and facemasks in situations where spatter and aerosols were generated. It appeared that most the dental surveyed practiced personal hygiene by routinely hand washing using antibacterial soap as shown in (figure 5).



(Figure 3) Sterilization and disinfection of laboratory equipment and materials.



(Figure 4) The general environment.



(Figure 5) The personnel protective facilities.

## DISCUSSION

Around 25 million items of service claims had been reported from general dental practitioners each year and of these, approximately 4.5 million claims involved work by dental technicians. Therefore, the spread of infections through the dental laboratory is a matter of concern and the safety of the dentist, technician and patient is only ensured through confidence that both professionals use proper procedures in a correct manner<sup>(10)</sup>. Various authoritative bodies such as the British Dental Association and the Dental Laboratory Association to clarify procedures to ensure the safety of patient and dental technicians have issued guidelines. This study was designed to evaluate the attitude and behavior of commercial dental laboratory workers in Misurata city about cross infection control hazards.

### Management:

Employers have the legal responsibility to reduce the spread of infection within the dental laboratory. Therefore, having a documented procedure for infection control will provide proper education and training as well as to aid compliance of all employees to routine cross infection control procedures. This survey demonstrated that only 20% of the dental laboratories in Misurata city were adhering to the stated recommendations from meeting. According to the Dental Laboratory Association guidelines, all dental laboratories and healthcare workers must have a documented procedure for infection control that entitles all staff to be immunized against hepatitis B and receive appropriate training in all aspects of infection control. This survey demonstrated that only 20% of the dental laboratories in Misurata had a staff policy, along with 38.5% immunized against hepatitis B and only a small proportion (10%) had received education and training regarding infection control.

### Facilities:

To prevent contaminated material from being recirculated in the laboratory or carried to patient areas, dental personnel should make sure that all lathes, grinders and lab hand pieces are connected to or used near a dust chip evacuation system. Within the dental laboratory there is evidence to indicate that material such as pumice is frequently contaminated with oral and non-oral bacteria including (*streptococci*, *staphylococci* and *enterics/coliforms* for

example *Pseudomonas species* and *Actineobacter species*). These organisms can theoretically cause infection, particularly in immunocompromised patients.

From our study it appeared that the majority of the laboratories did not clean or disinfect pumice daily even though the Dental Laboratory Association recommend the change of pumice and the disinfecting of the pan as well as the use of a liquid disinfectant as a mixing medium (5 parts sodium hypochlorite to 100 parts distilled water)<sup>(11,12)</sup>.

### Equipment:

Instruments and equipment used in the dental laboratory should be disinfected and sterilized on a regular basis. This will place more barrier in the path of possible cross contamination and provide less chance of introducing laboratory cross contamination during the production cycle. However, the vast majority of the laboratories did not accomplish this and this may reflect the lack of such recommendations in the Dental Laboratory Association policy. The findings of literature review revealed that all study casts examined could be a potential source of microbial contamination (even from the environment or air). Therefore, it could be a risk to the laboratory workers and as a consequence the dental staff or the patient either from direct or indirect methods. Following our literature review, the possible methods of disinfecting study casts include:

- 1- Clear slurry water (saturated calcium sulphate) with disinfectant causes no damage to surface detail.
- 2- Spraying the cast with iodophor or chlorine products.
- 3- placing the casts in a microwave.

### Environment:

Although it is apparent knowledge not to eat, drink or smoke in the laboratory, the occupational healthcare and safety organizations including the Dental Laboratory Association permit eating, drinking or smoking in the working areas. Most of the laboratories in Misurata did not comply with this Recommendation.

### Personnel:

In the laboratory, contaminated items are received and dealt with using equipment, which generate aerosols and spatter. There is therefore a need to wear protective clothing. In this section of the questionnaire

we found that most of the laboratories 45-67.5% practiced a healthy attitude when concerning only on safety precautions. Even though there are areas of concern in the breakthrough of the infection control cycle within the laboratory, the knowledge of the need and advantages of wearing personnel protective equipment as well as hand washing with soap routinely throughout the day were never the less understood by the majority.

#### CONCLUSION

There seems to be a definitive need to provide and distribute formal and obligatory standard of current infection control guidelines and manuals to the dental laboratories.

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# EVALUATION OF ANTIMICROBIAL PROPHYLAXIS USAGE IN THE SURGICAL PROCEDURES

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## ABSTRACT

Surgical site infections (SSIs) are the most common postoperative complications and represent a significant burden in terms of patient morbidity, mortality, and health costs. Choice of regimen, time of administration, and duration of antibiotic prophylaxis were inappropriate in 25-50% of cases from previous studies. Therefore, the main aim is to evaluate the appropriateness of antimicrobial prophylaxis use in surgery at the Misurata Central Hospital (MCH). The study included 247 surgical inpatients of any age, who were admitted and discharged from surgical departments: gynecology and obstetrics, orthopedics, and general surgery in the Misurata Central Hospital (MCH) over a period of six months from (1<sup>st</sup> January to 30<sup>th</sup> June 2016). The total of 247 patients underwent surgery and administered antimicrobial prophylaxis in the perioperative period. Appropriateness of antimicrobial prophylaxis was evaluated according to published guidelines of the American Society of Health-System Pharmacists (ASHP) for every aspect of antibiotic prophylaxis. All data were coded and analyzed using statistical software (Stat View version 5.1). The study showed 94.3% of prescribed and administered prophylactic antibiotics were in accordance with the American Society of Health-System Pharmacists (ASHP) guidelines regarding prophylaxis indication. The total compliance for the studied parameters was (3.2%), only (10.5%) had appropriate antibiotic selection, and (50.6%) of patients received antibiotic in appropriate duration. The general surgery department had higher total adherence to the ASHP guidelines ( $P=0.006$ ). The gynecology department had much compliance regarding duration of antibiotic use ( $P = 0.00001$ ); however, general surgery had higher proper antibiotic selection ( $P = 0.0001$ ). A lack of concordance between the use of antimicrobial prophylaxis and prescribing guidelines was identified in this study at the MCH.

**KEY WORDS:** Adherence, Antibiotic prophylaxis, Guidelines, Surgery.

## INTRODUCTION

Surgical site infections (SSIs) are the most common nosocomial infections among surgical patients<sup>(1-3)</sup>. They represent 14-16% of hospital-acquired infections<sup>(3)</sup>, up to 2% to 5% of patients undergoing clean extra abdominal operations and up to 20% of patients undergoing intra-abdominal operations<sup>(4,5)</sup>. They associated with increased healthcare cost, use of antimicrobial agents, prolonged hospitalization, permanent disability or mortality<sup>(1)(3,6)</sup>. Mortality rates are 2-3 times higher in patients in whom SSIs develop compared with un-infected patients<sup>(7)</sup>.

The risk of SSIs depends on patient-related factors such as age, nutritional status and co-existing infection and surgical factors, such as duration of surgery and class of wound (clean, clean-contaminated, contaminated, and dirty-infected)<sup>(7)</sup>. Consequently, the basic principle of Surgical Antimicrobial Prophylaxis (SAP) use is to achieve adequate serum and tissue drug levels that exceed the total duration of operation, and prevent the occurrence of surgical site infections<sup>(7,8)</sup>. In addition, the efficacy of surgical antimicrobial prophylaxis depends on several factors, including selection of appropriate antibiotic, timing of administration, dosage, duration, and route of administration<sup>(1)</sup>. Furthermore, adequate use of pre-operative antibiotic prophylaxis can reduce the rate of SSI in up to 50%<sup>(2)</sup>.

Because, there was a wide variation of overall compliance towards surgical antimicrobial prophylaxis

(SAP) guidelines ranging from 0 to 71.9% from previous studies<sup>(1)</sup>. The American Society of Health System Pharmacists (ASHP) has developed therapeutic guidelines on antimicrobial prophylaxis in surgery<sup>(9)</sup>. According to the ASHP guidelines, antibiotic should be administered within 1 hour before incision to achieve prophylactic level during surgery and optimize efficacy<sup>(9)</sup>. For vancomycin, the infusion should begin within two hours before incision, and doses should be repeated during operation if the operation is still in progress two half-lives after the first dose<sup>(9)</sup>. Antibiotic administration should be discontinued within 24 hours after the end of surgery to prevent emergence of resistance<sup>(9)</sup>. Finally, inexpensive, non-toxic, and narrow spectrum antibiotic should be used; therefore, intravenous (I.V) cefazolin is recommended for the most of surgical procedures at recommended dose 2gm for patients < 120 gm and 3gm for  $\geq 120$ kg (9). Cefoxitin is recommended for appendectomy and colorectal procedures and vancomycin is reserved for patients with beta lactam allergy<sup>(9)</sup>.

Therefore, the main aim of this study was to determine retrospectively the appropriateness of antimicrobial prophylaxis use in surgery at the Misurata Central Hospital (MCH) through assessing adherence to the American Society of Health-System Pharmacists (ASHP) therapeutic guidelines.

## MATERIALS AND METHODS

### Study design and setting:

This was a clinical type, conducted as a descriptive, retrospective study in the largest referral and teaching hospital in Misurata, which is the Misurata Central Hospital (MCH) over a period of six months from 1<sup>st</sup> January through 30<sup>th</sup> June 2016. The study

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involved the surgical patients who were admitted to three surgical departments, gynecology and obstetrics, general surgery and orthopaedic department.

#### Selection of patients:

The study was carried out on surgical inpatients of any age who discharged from the MCH over a period of study. The design of the study included 247 patients in total who were consecutively selected from the total surgical list. There was no restriction to type of surgery, or whether procedures were open or laparoscopic, elective or urgent, and clean or clean contaminated. The total 247 patients underwent surgery and received preoperative antibiotics were compared with the published therapeutic guidelines of the ASHP 2013<sup>(9)</sup>.

#### Inclusion and exclusion criteria:

Any patient underwent surgery and received surgical antimicrobial prophylaxis for clean surgery which is operative procedure does not enter into a normally colonized viscus or lumen of the body<sup>(10)</sup> or clean contaminated surgery in which the operative procedure enters into colonized viscus or cavity of the body, but under elective and controlled circumstances<sup>(10)</sup> in the study period was included in study. Patients who received antibiotics to treat infection prior to surgery were excluded, as were patients for whom it was not possible to determine whether antibiotic was given as treatment or prophylaxis. In addition, contaminated and dirty surgeries were excluded because antibiotics would be routinely administered as therapeutic intervention. Furthermore, complicated appendicitis such as perforated or gangrenous was also excluded.

#### Data collection:

Data were collected directly from medical files of patients and entered on data collection form. The data included patient demographics, Type of surgery, and antibiotic therapy received such as type of antibiotic, dose, and dose interval, route of administration, initiation time, and duration of prophylaxis. Compliance with recommendations of the ASHP therapeutic guidelines was assessed for every aspect of antibiotic prophylaxis.

#### Statistical analysis:

All variables were entered and analyzed by using statistical software (Stat View version 5.1). The results were presented as mean  $\pm$  standard deviation and percent (%) where applicable. Comparison of qualitative data was performed by Chi-square test. Assessment of surgeon adherence to the ASHP guidelines (compliance versus noncompliance) was performed. A significant difference was considered when the P value was less than 0.05.

## RESULTS

A total of 247 (17 male & 230 female) patients were included in the study. Mean age of patients was 31.8  $\pm$  14.4 years. The great majority (60.7%) of patients had an elective procedure while (39.3%) were operated on an emergency basis. Most patients were admitted under General surgery department (45.3%).

Caesarean section was the most frequent surgical procedure performed (40.5%). (Table 1) shows the demographic data of patients and distribution of procedures according to surgical specialty.

(Table 1) Characteristics of the surgical patients received prophylactic antibiotics

Characteristics		Value (%)
Age (years)	Mean	31.8 $\pm$ 14.4
	Range	3-89
Gender	Male	17 (7)
	Female	230 (93)
Length of hospital stay (Days)	Mean	2.9
	Range	0-23
Surgical wards	General surgery	(45.3)112
	Gynecology and obstetric	(40.5)100
	Orthopaedic	(14.2)35
Operation types	Cesarean section	(40.5)100
	Cholecystectomy	(30.4)75
	Appendectomy	(12.6)31
	Fracture reduction and fixation	(10.9)27
	Herniotomy (herniorrhaphy)	(2.1)6
	Others	(3.1)8
Electivity of surgical operation	Elective	150 (60.7)
	Emergent	97 (39.3)
Wound class	Clean	14 (6)
	Clean contaminated	233 (94)
Surgery type	Open	173 (70)
	Laparoscopic	74 (30)

According to the ASHP guidelines, prophylactic antimicrobials were indicated and administered in (94.3%) of all cases; however, were administered inappropriately in (5.6%) patients such as herniorrhaphy and clean operations involved hand. A total number of 455 antibiotics were prescribed and administered in the current study. The most commonly prescribed and administered antibiotic was ceftriaxone (89.4%) followed by metronidazole (71.6%) and co-amoxiclav (Amoxicillin + clavulanic acid) (19%). Ceftriaxone was appropriate in 26 of 247 evaluated surgical procedures (10.5%) and the dose and dosing interval were appropriate.

In addition, metronidazole and co-amoxiclav were the most antibiotics frequently used inappropriately. Furthermore, 202 patients for whom a single antibiotic was indicated, 150 (74.2%) received two or three antibiotics.

(Table 2) shown surgical prophylactic antibiotics used in three wards. Regarding the duration of prophylaxis in current study, it was consistent with the ASHP recommendation for 125 (50.6%). In 94 (38%) procedures, duration was more than 24 hours, and mean duration of prophylaxis was 4.5  $\pm$  days. In

14 (6%) procedures, the duration of prophylaxis was less than 24 hours.

**(Table 2)** Antimicrobials used in different wards for surgical prophylaxis (n=247)

Type of antibiotic	Surgical wards			Total	Rate of compliance With ASHP guidelines (%)
	Orthopaedic	Gynecology and obstetric	General		
Single					
Ceftriaxone	30	0	29	59	10.5
Co-amoxiclav	0	0	4	4	0
Two drug combinations					
Ceftriaxone+Metronidazole	0	59	73	132	0
Co-amoxiclav+Metronidazole	0	18	2	20	0
Ceftriaxone+Cloxacillin	5	0	0	5	0
Cefotaxime+Metronidazole	0	2	0	2	0
Ceftriaxone+Coamoxiclav	0	0	1	1	0
Three drug combinations					
Ceftriaxone+Metronidazole + Co-amoxiclav	0	21	2	23	0
Ceftriaxone+Metronidazole + Gentamicin	0	0	1	1	0
<b>Total</b>	<b>35</b>	<b>100</b>	<b>112</b>	<b>247</b>	<b>10.5</b>

The overall rate of compliance with the ASHP guidelines was (3.2%) and the remaining (96.7%) of cases did not comply as in (table 3).

**(Table 3)** The percentage of compliance with the ASHP therapeutic guidelines of antibiotic prophylaxis in surgery in study

Variable	No (%)
Appropriate indicatio	233 (94.3)
Appropriate antibiotic choice	26 (10.5)
Appropriate antibiotic dose and dose interval	43 (17.4)
Appropriate duration of prophylaxis	125 (50.6)
Overall compliance	8 (3.2)

The main reasons for noncompliance with the ASHP guidelines were inappropriate selection of antibiotics (89.4%), inappropriate dosing (83%), inappropriate duration of prophylaxis either prolonged duration (38%), or too short duration (6%) and improper indication (5.7%). Regarding surgeons adherence to the ASHP guidelines, there was statistical difference in the compliance rate between three different surgical specialties ( $P = 0.006$ ). In addition, the gynaecology and obstetric department had higher compliance regarding duration of antibiotic use ( $P = 0.00001$ ); however, the general surgery department had much proper antibiotic selection and dosing ( $P = 0.00001$ ). There was no statistical difference in the adherence to the ASHP guidelines between clean and clean contaminated surgeries ( $P = 0.48$ ). Furthermore, the compliance rate was significantly higher for elective and laparoscopic procedures ( $P = 0.02, 0.00001$ ) respectively as in (table 4, 5).

**(Table 4)** Assessment of surgeon's adherence to antibiotic prophylaxis guidelines

	Compliance (%)	Noncompliance (%)	P value
<b>Wound class</b>			0.48
Clean	0	14 (6)	
Clean contaminated	8 (100)	225 (94)	
<b>Surgical procedures</b>			0.006*
General surgery	8 (100)	104 (43.5)	
Gynecology surgery	0	100 (41.8)	
Orthopaedic surgery	0	35 (14.6)	
<b>Electivity of surgical procedure</b>			0.02*
Elective	8 (100)	142 (59.4)	
Urgent	0	97 (40.5)	
<b>Surgery type</b>			0.00001*
Open	0	173 (72.4)	
Laparoscopic	8(100)	66 (27.6)	

*P value\* ( $P < 0.05$ ): indicates the difference is statistically significant*

**(Table 5)** Compliance with antibiotic prophylaxis according to surgical department

	Compliance (%)	Noncompliance (%)	P value
<b>Appropriate duration of prophylaxis</b>			0.00001*
General surgery	41 (32.8)	71 (58.2)	
Gynecology surgery	74 (59.2)	26 (21.3)	
Orthopaedic surgery	10 (8)	25 (20.4)	
<b>Appropriate antibiotic selection</b>			0.00001*
General surgery	26 (100)	86(38.9)	
Gynecology surgery	0	100 (45.2)	
Orthopaedic surgery	0	35 (15.8)	
<b>Appropriate dosing</b>			0.00001*
General surgery	27 (62.7)	85(41.6)	
Gynecology surgery	0	100 (49)	
Orthopaedic surgery	16 (37.2)	19 (9.3)	

*P value\* ( $P < 0.05$ ): indicates the difference is statistically significant.*

## DISCUSSION

This study reports adherence to ASHP surgical antibiotic prophylaxis guidelines at three surgical departments in the MCH. The results can provide evidence for recommendations that may help to improve health care. In current study, adherence to ASHP guidelines was very low (only eight cases). Low adherence was also shared by other studies; for example, The Iranian study found only three surgical procedures of the 1000 patients were adherent to all parameter of prophylaxis guidelines with varying degrees of compliance in different parameters<sup>(11)</sup>. In addition, the commonest reasons for noncompliance were inappropriate antibiotic selection (89.4%).The third generation cephalosporins were reported as the first used regimen in this study and this finding was similar to study via Aljarari and Pella (2013)<sup>(3,5)</sup>. Broad spectrum antibiotics for surgical prophylaxis were recommended mainly for severe infection or in

acute infection while waiting for the results of cultures<sup>(10)</sup> and excessive use of broad spectrum antibiotics increase risk for resistance, adverse effects and healthcare cost<sup>(12)</sup>. The main reason could be easy availability of drug in the hospital pharmacy according to Aljarari and Pella (2013)<sup>(3)</sup>.

The second reason for noncompliance was prolonged duration of prophylaxis in this study. Extended prophylaxis had been shown no benefit and is potentially harmful due to the development of drug toxicity, superinfection, and bacterial resistance<sup>(12)</sup>. The adherence rate was similar between clean and clean contaminated surgery ( $P = 0.48$ ) in this study. This finding was different from previous studies; as compliance rate was significantly higher for clean surgery than clean contaminated surgeries<sup>(10,12)</sup>. The general surgery department had higher adherence to the ASHP therapeutic guidelines ( $P = 0.06$ ). In addition, general surgery department showed a better adherence in selection of antibiotics ( $P = 0.00001$ ), however; the gynaecology and obstetric department had higher compliance regarding duration of antibiotic use ( $P = 0.00001$ ). This finding was similar to studies conducted in and Turkey and Palestine in 2003, 2014 respectively<sup>(7,13)</sup>.

The limitations of the current study include the involvement of small number of patients which did not give complete overview of the compliance rate among the different departments. Moreover, the current study did not analyze one important element of surgical antibiotic prophylaxis the timing of antibiotic administration before incision due to lack of data. However, this is not crucial in the results as being noncompliant with one element of the prophylaxis is considered as a guidelines deviation.

### CONCLUSION

The study highlighted limitations in prescription of prophylactic antimicrobial agents in surgical procedures according to the published therapeutic guidelines, as defined by the ASHP guidelines. Surgical prophylaxis was inappropriate in terms choice of antimicrobial agent, prolonged duration of prophylaxis following surgery. Interventions are warranted to promote the development, dissemination and adoption of evidence-based guidelines for antimicrobial prophylaxis.

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**SYNTHESES AND ANTIMICROBIAL ACTIVITY  
OF NEW CYCLOHEXAPYRAZOLINE DERIVATIVE**

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**ABSTRACT**

A new cyclohexapyrazoline compound was synthesized by reacting 2,6-bis(4-(dimethylamino)benzylidene) cyclohexanone (I) with hydrazine hydrate in methanol utilizing the microwave technique. The structure of the new cyclohexapyrazoline compound was confirmed by elemental and spectral analysis. Finally, the antimicrobial activity of the new cyclohexapyrazoline compound (II) was studied.

**KEYWORDS:** Michael addition; microwave irradiation; pyrazoline; antimicrobial activity.

**INTRODUCTION**

Pyrazolines are a form of heterocyclic compounds that have much attention, due to their biological activity<sup>(1)</sup>. Pyrazoline derivatives have antifungal<sup>(1-3)</sup>, antiviral and antibacterial<sup>(4-6)</sup>, and are also known for their chromosomal properties<sup>(7)</sup>. Optimizing synthetic procedures for pyrazolines consisting of different substituents an objective for of obtaining some new pyrazoline compounds with potentially enhanced properties. The development of new, fast and clean synthetic routes toward libraries of pyrazoline heterocycles is interest to the chemists. They have been reported in literature survey for the design and development of novel pyrazoline rings via multistep preparations<sup>(8-10)</sup>. The common synthesis of the pyrazoline is through Michael 1,4-addition of an  $\alpha$ ,  $\beta$ -enone with a hydrazine derivative<sup>(8)</sup>. The preparation of different pyrazoline derivatives, has been a developing field within the regality of heterocyclic chemistry for the past few years, because of their readily accessibility and the broad spectrum of biological activity of the products<sup>(11,12)</sup>. These results led us to synthesize different pyrazoline and discovering simple procedures such as irradiation chemistry<sup>(8,10,11)</sup>, since it offers a new clean approach towards the synthesis of organic compounds. In the recent years, the ultrasonic microwave methods have become widely used in organic synthesis<sup>(10-14)</sup>, This technique has been employed, not only to minimize reaction times, but also to improve yields in a large variety of heterocyclic compound, and ease of control<sup>(13)</sup>. Therefore, the development of these compounds for the use in different applications with improved characteristics is an important goal. In this present study, pyrazoline compound derived from dibenzylidene cyclohexanone with hydrazine hydrate was prepared. The effect of pyrazoline structure on the biological activities of this resin against bacteria and fungi have also been examined.

**EXPERIMENTAL**

**Instrumentation:**

Melting points were determined on a Perkin-Elmer 240°C electro thermal melting point apparatus and

are uncorrected. Elemental analyses were performed on Elementar Vario EL III instrument. Infrared spectra were recorded on a Shimadzu 2110 PC spectrophotometer with KBr pellets. The <sup>1</sup>H-NMR spectra were recorded on a GNM-LA 400 MHz NMR spectrophotometer at room temperature in DMSO or CHCl<sub>3</sub> using TMS as the internal reference and the chemical shifts were recorded by ppm. Microwave irradiation carried out using a microwave closed system (GEEPAS. Model No: GMO1874.27LD,50Hz).

**Reagents and solvents:**

4-(dimethylamino)benzaldehyde and hydrazine hydrate were purchased from (Aldrich) and used without further purification. Methanol and all other solvents were of high purity and were further purified by standard methods<sup>(15)</sup>.

**Syntheses of pyrazoline compound:**

**Syntheses of 2,6-bis (4-(dimethylamino) benzylidene) cyclohexanone:(I)**

The 2,6-bis (4-(dimethylamino) benzylidene) cyclohexanone (I) was prepared as described in the literature<sup>(16)</sup>.

**Syntheses of 4-((3-(4-(dimethylamino) phenyl)-,3,3a,4,5,6-hexahydroindazol-7-ylidene) methyl)-N,N-dimethylbenzenamine:(II)**

Equimolar amounts 0.01 mole (3.60g) of compound (I), 0.05 mole (2.91 g) of hydrazine hydrate (98%) and dry Teflon vessels. In this case a minimum amount of methanol (10–15 ml) was used to dissolve the reaction mixture. This mixture was subjected to microwave irradiation at 400 W power for about 2 min with maximum heating of 60 °C. The completion of reaction was checked by TLC using mobile phase dichloromethane/ethyl acetate (7/2). The reaction mixture was allowed to cool to room temperature and the crude solid product was collected through vacuum filtration and washed several times with ethanol, and finally recrystallized from absolute ethanol to yield (85%). The pure product was conserved in a dark bottle in the refrigerator, mp. 270°C. Calculated composition of C<sub>24</sub>H<sub>30</sub>N<sub>4</sub>: C, 77.00; H, 8.02; N, 14.97 % Found: C, 77.16; H, 8.11.; N, 14.73 %. IR(KBr): at 1610 cm<sup>-1</sup> (s, C = C), at 1630 cm<sup>-1</sup> (s, C = N), at 2850-2940 (C-H of cyclohexapyrazoline) and at 3220 cm<sup>-1</sup> (br, N-H stretching). <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): at 8.00-7.00 (m, 8H of Ar-H ), at 5.95 (s, H of CH=C), at 5.50 (s, H of N-H pyrazoline), at 4.3 (d, H of CH-N pyrazoline), at 3.9 (d, H of CH-C hexapyrazoline), at

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3.30 (*d*, 12H of CH<sub>3</sub>-N-CH<sub>3</sub>), at 2.70 (*t*, 4H of 2CH<sub>2</sub> cyclohexapyrazoline), and at 1.20 (*t*, middle, 2H of CH cyclohexapyrazoline).

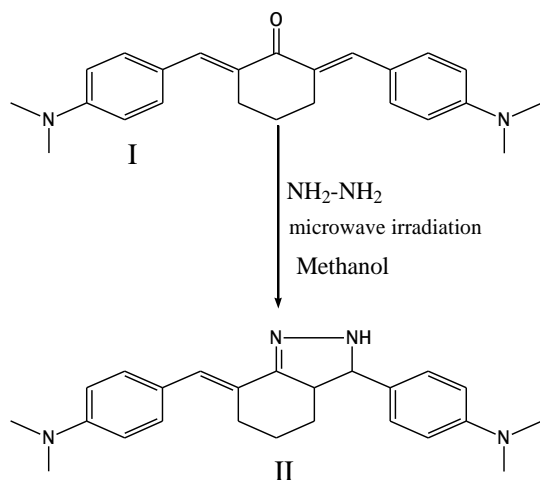
#### Antimicrobial activity screening of pyrazoline compound II:

The antibacterial activity of the synthesized pyrazoline compound was tested against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli* using nutrient agar medium. The antifungal activity of the compounds was tested against *Candida albicans* and *Aspergillus niger* using sabouraud dextrose agar medium. Both activities were performed by paper disc diffusion method<sup>(17)</sup>. The sterilized (autoclaved at 120°C for 30 min) medium (40–50°C) was inoculated (1 mL/100 mL of medium) with the suspension (105 cfu/mL) of the microorganism (matched to McFarland barium sulphate standard) and poured into a petri dish to give a depth of 3–4 mm. The paper impregnated with the test compounds (50 µg/mL in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37°C for 24 and 48 h for antibacterial and antifungal activity respectively. Ciprofloxacin and ketoconazole were used as standard for antibacterial and antifungal activity respectively.

## RESULTS AND DISCUSSION

#### Syntheses of pyrazoline compound:

In 1964 and by using Michael condensation, Sammour *et al* [18] have reported some substituted cycloalkanones with phenyl hydrazine. In another study [19], doubly unsaturated ketones such as dibenzylideneacetone showed the two double bonds in dibenzylideneacetone underwent Michael condensation independent on each other. In this work, a new heterocyclic compound based on cyclohexapyrazoline moiety was prepared by a condensation reaction between of 2,6-bis(4-(dimethylamino) benzylidene) cyclohexanone (I) and hydrazine (figure 1).



(Figure 1) Syntheses of pyrazoline compound II.

The IR data for the compound II showed the disappearance of the C=O group of starting material and the absorption band appearance of the C=N group

appeared at 1630 cm<sup>-1</sup>. The N-H group of the cyclohexapyrazoline was also seen at 3220 cm<sup>-1</sup>. This indicated that the compound II was formed. The <sup>1</sup>H-NMR data confirmed the structure formula of the resulting pyrazoline derivative II.

#### Antimicrobial activity:

The *in vitro* antimicrobial activity of the synthesized pyrazoline compound was performed using the disk diffusion method with different Gram positive (*Streptococcus pyogenes*, *Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria as well as fungal strains (*Candida albicans*, *Aspergillus niger*). The antimicrobial activity potentials were qualitatively assessed by the inhibition zone diameters. Ciprofloxacin and ketoconazole were used as positive controls for bacteria and fungi respectively. In a preliminary assay, the tested pyrazoline compound showed activity against all tested microbial strains, and the obtained results were presented in table 1. The tested pyrazoline compound displayed a variable level of antimicrobial activity on all the tested strains. It can be clarified from table 1 that the highest antibacterial activity was observed against *Streptococcus pyogenes* with zone of inhibition of 18 mm followed by *Staphylococcus aureus* and *Escherichia coli* exhibiting a maximum zone of inhibition of 16 mm. It was interesting to observe that these activities were comparable with the standard drugs ciprofloxacin and ketoconazole demonstrating a zone of inhibition of 25 mm and 21 mm respectively.

(Table 1) Antimicrobial activity of the tested pyrazoline compound (expressed as the diameter of the inhibition zone<sup>a</sup>)

Name of compound	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>E. coli</i>	<i>Streptococcus pyogenes</i>	<i>Staphylococcus aureus</i>
Pyrazoline II	05	07	14	18	16
Ciprofloxacin	13	10	20	25	22
Ketoconazole	11	14	19	20	21

<sup>a</sup> Average of three observations;

Inhibition zone in mm (Disc diameter 6 mm)

The moderate antibacterial activity was noted with the pyrazoline moiety based on dibenzylidene cyclohexanone which may be attributed to the conformation of the heterocyclic pyrazoline ring II. The antifungal activity of the pyrazoline compound II was studied for the two pathogenic fungi. It was observed that pyrazoline compound II had the lowest activity against *C. albicans* and *A. niger*

exhibiting a maximum zone of inhibition of 7 mm. Moreover, the tested pyrazoline compound exhibited moderate antibacterial activity against all three strains of bacteria, but the activity was less against the two pathogenic fungi (*C. albicans* and *A. niger*).

## CONCLUSION

New compound of dibenzylidene cyclohexanone con-

taining pyrazoline moiety was synthesized *via* ultrasonic microwave technique. The structure of the new pyrazoline compound was confirmed by spectral analysis. The synthesized pyrazoline compound could be considered as a moderate to good antimicrobial agents.

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# KNOWLEDGE AND PRACTICE OF STANDARD PRECAUTIONS AMONG NEONATAL INTENSIVE CARE UNIT HEALTHCARE WORKERS OF MISURATA TEACHING HOSPITAL, LIBYA

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## ABSTRACT

Nosocomial infections persist as a major problem in neonatal intensive care units. Neonates in the NICU are vulnerable to many nosocomial infections from multiple devices for treating or monitoring their care. Thus, healthcare workers should have sound knowledge and strict adherence to infection control standard precautions. To assess the knowledge and practice of standard precautions among neonatal intensive care unit (NICU) healthcare workers in Misurata teaching hospital. A descriptive cross-sectional study was carried out on a sample of 28 consenting doctors, nurses and laboratory scientists in Neonatal Intensive Care Units of Misurata Teaching Hospital, Libya, during the period from March to May 2015. Data was collected through two tools: a) Structured questionnaire to assess knowledge level about standard precautions; and b) Performance observational checklist to assess practice of standard precautions. Data was analyzed using SPSS version 18. Majority of the studied HCWs are females (71.4%), belonged to the age group of 20-30 years (57.1%), with work experience less than 10 years (64.3%) and only 21.4% received infection control training. The overall mean knowledge score toward standard precautions is 68.4%, but mean practice score is 47.4%. And all scores are significantly lower among those who were non-trained. 89.3% of staff had good awareness in hand hygiene moments, 53.6% aware of hand washing steps, but only 32.1% actually practiced it right. While 85.7% of the staff had knowledge in use of personal protective equipments and fresh gloves, only 67.8% wore in practice. 53.6% knows about oral health care practices protocol, only 35.7% actually did it. 75% of the staff are aware of sterile technique in CVC care, 57.1% followed catheter care practices as per protocol. 64.3% are aware how to dispose wastes and sharps off in a right way, 46.3% did it frequently. While 85.7% are satisfied about instruments sterilization, only 39.3% of HCW reported that NICU environment is clean. There is inadequate knowledge and poor compliance with standard precautions of infection control among NICU health-care staff in Libya. It is very important to develop infection control policy and protocols that strengthen the training of HCW in standard precautions to improve not only their knowledge but also their practice.

**KEYWORDS:** Knowledge, Practice, Standard precautions, Neonatal Intensive Care Unit.

## INTRODUCTION

Hospital-acquired infection or Nosocomial infection refers to infections that may be acquired during the process of care and not manifested at the time of admission to a health care facility<sup>(1)</sup>. It is one of the leading causes of increased morbidity and mortality; longer duration of hospital/ICU stay; increased severity of the underlying illness; increased utilization of devices for monitoring and treatment; and increased cost of treatment<sup>(2)</sup>.

Hospital-acquired infections are a common occurrence in the neonatal intensive care unit (NICU). NICU is considered one of the high-risk areas in hospital that encounter high rate of infection and mortality<sup>(3)</sup>.

Neonates are more susceptible to infection because their host defense mechanisms are not mature. Also, they occupy an environment in which invasive interventions and antibiotics are frequently used. That often permit the invasion of common nosocomial pathogens, and the close proximity of patients in many NICUs facilitates transfer of organisms from patient to patient<sup>(4)</sup>.

It has also been suggested that the hands of healthcare workers play a significant role in the

transmission of nosocomial infection in NICUs, as *Pseudomonas aeruginosa*, a common nosocomial pathogen among neonates. However, in NICUs, modern neonatal nursing emphasizes the concepts of minimal handling coupled with clustering of nursing care<sup>(5)</sup>.

The implementation of infection prevention and control guidelines is important for the improvement of quality care in hospitals. Standard precautions are the basic infection control precautions that should be followed in any setting where health care is delivered<sup>(6)</sup>.

It is pertinent to mention here that little resources are required to implement standard precautions<sup>(7)</sup>. Hand hygiene practices of health care workers is the most important and an effective procedure in preventing nosocomial infection<sup>(8)</sup>. The recommendation on hand hygiene has recently been updated, and hand-washing has been replaced by hand rub as the standard of care<sup>(4)</sup>.

Implementation of standard precautions is dependent on the level of knowledge and compliance of healthcare workers, with a significant direct impact on the patient and HCW safety<sup>(9)</sup>. Failure to comply with policies and procedures that support the reduction of hospital acquired infections (HAIs) is a recognized problem. According to Centre for Disease Control (2002), limited knowledge, lack of facilities and poor working environment are commonly cited

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as barriers to compliance<sup>(7)</sup>. So, we conducted this study with the aim to assess the knowledge and practices of standard precautions among healthcare workers in the neonatal intensive care unit of Misurata teaching hospital.

## METHODS

### Study setting:

This study was conducted as an observational cross-sectional study at the neonatal intensive care unit of Misurata teaching hospital in Libya, during the period from March to May 2015.

### Study subjects:

Our study included 28 NICU health care workers, they were as follow: 11 nurses, 9 doctors, 5 house officers and 3 laboratory scientists.

### Ethical issue:

An informed consent was obtained from all participants in this study.

### Data collection:

The data was collected data using two tools: structured questionnaire: containing questions about: (i) Demographic characteristics: age, sex, profession, work experience and IC training Questions to assess their knowledge level about standard precautions.

They included knowledge of hand hygiene moments, hand washing steps, knowledge about use of personal protective equipment's and fresh gloves, oral health care practices protocol, sterile technique in CVC care, proper wastes and sharps disposal, instruments sterilization and environmental sanitation. (ii) Two questions about their satisfaction about instruments sterilization and environmental sanitation. B) Performance observational checklist: to assess their practice of standard precautions.

### Statistical analysis:

Data was analyzed by SPSS software version 18 and the results was summarized, presented and displayed as frequencies and percentage in suitable tables. Statistical analysis of qualitative data was performed using Chi-square test, while fisher exact test was used if there is an expected value in a cell < 5. Results were accepted as significant when ( $p < 0.05$ ).

## RESULTS

The Majority of the studied HCWs were females (71.4%), belonged to the age group of 20-30 years (57.1%), with experience less than 10 years (64.3%) and only 21.4% of them had received infection control training. They were as follow 39.3% nurses, 32.2% doctors, 17.8% house officers and 10.7% laboratory scientists (table 1).

Among the studied NICU health care workers, the overall mean knowledge score toward standard precautions was 68.4% and the knowledge scores are significantly higher among HCWs who had received training on infection control than that among those who were not trained (91.7% and 63.1% respectively).

**(Table 1)** Relevant Characteristics of the Studied NICU Health Care Workers.

Relevant Characteristics	Total NICU HCWs N. (28)	
	Freq.	%
<b>Age</b>		
20 - 30	16	57.1
> 30 - 40	8	28.6
> 40	4	14.3
<b>Sex</b>		
Male	8	28.6
Female	20	71.4
<b>Type of healthcare worker</b>		
House officers	5	17.8
Doctor	9	32.2
Nurse	11	39.3
Laboratory scientists	3	10.7
<b>Work experience</b>		
< 10	18	64.3
≥ 10	10	35.7
<b>Received IC training</b>		
Yes	6	21.4
No	22	78.6

89.3% of staff had good awareness in hand hygiene moments and 53.6% were aware of hand washing steps. While 85.7% of the staff had knowledge in use of personal protective equipments and fresh gloves, 53.6% knows about oral health care practices protocol, 75% of the staff are aware of sterile technique in CVC care, 64.3% were aware how to dispose wastes and sharps off in a right way, 71.4% had knowledge about instruments sterilization and only 53.6% of HCW reported knowledge about proper environmental sanitation (table 2).

**(Table 2)** Knowledge of Standard Precautions among the Studied NICU Health Care Workers.

Knowledge of Standard Precautions	Trained 6 (21.4%)		Un-trained 22 (78.6%)		Total NICU HCWs N. (28)		P. value
	N.	%	N.	%	N.	%	
Hand hygiene moments	6	100	19	86.4	25	89.3	0.94
Hand washing steps	6	100	9	40.9	15	53.6	0.013*
Use of personal protective equipments	6	100	18	81.8	24	85.7	0.71
Oral health care practices protocol	5	83.3	10	45.4	15	53.6	0.049*
Sterile technique in CVC care	5	83.3	16	72.7	21	75	0.52
Proper wastes and sharps disposal	6	100	12	54.5	18	64.3	0.024*
Instruments disinfection and sterilization	5	83.3	15	68.2	20	71.4	0.43
Environmental cleaning	5	83.3	10	45.4	15	53.6	0.049*
Mean Overall Knowledge	5.5	91.7	13.9	63.1	19.1	68.4	<0.001*

Majority of NICU health care workers are satisfied about instruments sterilization (85.7%), but only 39.3% of HCW reported that NICU environment is clean (table 3).

**(Table 3)** Satisfaction of NICU Health Care Workers about Instruments Sterilization and the Quality of Environmental Sanitation

Satisfaction	Total NICU HCWs N. (28)	
	N.	%
Instruments Sterilization	24	85.7
Quality of Environmental Sanitation	11	39.3

The overall mean practice score of standard precautions among NICU health care workers is 47.4% and the practice scores are significantly higher among

those who were trained on infection control than that among those who were not trained (76.2% and 39.6% respectively). Only 32.1% actually practiced hand hygiene as protocol, 67.8% wore protective equipments and fresh gloves in practice, 35.7% practice oral health care practices as per protocol, 57.1% followed catheter care practices as per protocol. 46.3% frequently dispose wastes and sharps off in a right way, 64.3% frequently use sterilized instruments and only 28.6% of HCW follow environmental cleaning as recommended (table 4).

**(Table 4)** Practice of Standard Precautions among the Studied NICU Health Care Workers

Practice of Standard Precautions	Trained 6 (21.4 %)		Un-trained 22 (78.6 %)		Total NICU HCWs N. (28)		P. value
	N.	%	N.	%	N.	%	
Hand hygiene steps	4	66.6	5	22.7	9	32.1	0.035*
Use of personal protective equipments	6	100	13	59.1	19	67.8	0.046*
Oral health care practices protocol	3	50	7	31.8	10	35.7	0.36
Sterile technique in CVC care	5	83.3	11	50	16	57.1	0.16
Proper wastes and sharps disposal	5	83.3	8	36.3	13	46.4	0.031*
Instruments disinfection and sterilization	5	83.3	13	59.1	18	64.3	0.27
Environmental cleaning	4	66.6	4	18.2	8	28.6	0.038*
Mean Overall Practice	4.6	76.2	8.7	39.6	13.3	47.4	<0.001*

## DISCUSSION

Nosocomial infections are being a common problem all over the world. It is a continuing problem in intensive care units causing increased morbidity, mortality and excess health care cost<sup>(4)</sup>. Therefore, increase knowledge and improve practical HCWs' skills can play important roles in preventing nosocomial infection. HCWs in neonatal intensive care units should practice standard precautions on a day-to-day basis as an integral part of patients' care<sup>(10)</sup>. Officials at the CDC estimate that one third of all nosocomial infection are caused by a lack of adherence to established infection control practices and standard precautions<sup>(4)</sup>. So, our study attempted to assess the level of knowledge as well as the practices of neonatal intensive care unit healthcare workers regarding standard precautions.

As demonstrated from the current study, nearly 57.1% of the studied HCWs aged between 20 to 30 years old. This finding is in concordance with that of Eskander et al<sup>(10)</sup> who studied knowledge and practices regarding standard precautions among Egyptian cancer hospital intensive care nurses, and founded that about two thirds of them was 20-30 years. Also it is similar to that of Sreedharan et al<sup>(11)</sup> in the United Arab Emirates and that of Efstathiou et al<sup>(12)</sup> which studied compliance of Cypriot nurses with standard precautions. While, Labrague et al<sup>(13)</sup>, found in their study that the mean age was slightly lesser. This young age of the studied sample reflect the ability to acquire knowledge and change their practices based on submission of IC training. In this

regards Alwutaib et al<sup>(14)</sup> revealed that older age is an important determinant of lower knowledge levels. Concerning gender, our study revealed the dominance of females among HCWs (71.4%), with work experience less than 10 years (64.3%). These findings are in agreement with that of Eskander et al<sup>(10)</sup>, Labrague et al<sup>(13)</sup>, Vaz et al<sup>(15)</sup>, and Hamid et al<sup>(16)</sup>, who founded also the dominance of females among their studied samples. As well, Eskander et al<sup>(10)</sup> and Kennedy et al<sup>(5)</sup>, found the great majority of their studied sample had work experience less than 5 years.

The present study revealed unsatisfactory level of knowledge among NICU health care workers. The overall mean knowledge score toward standard precautions is 68.4%. These results are in agreement with the findings of Eskander et al<sup>(10)</sup> and Qayyum et al<sup>(17)</sup> in their studies, who found poor knowledge about nosocomial infections and standard precautions measures among the studied HCWs. However, on contrary to our findings, Ibrahim et al<sup>(18)</sup>, revealed that majority of their studied group were aware of nosocomial infections transmission and standard precautions. In this regards Perry and Potter<sup>(19)</sup> concluded that increasing nurses' knowledge about standard precautions can intervene to prevent developing of infections.

In this study, 89.3% of staff had good awareness in hand hygiene moments and 53.6% were aware of hand washing steps, but only 32.1% actually practiced it right. A similar study conducted by Sharma et al<sup>(20)</sup> demonstrated that 86% was aware of all five

moments of hand hygiene and 80% knew all steps of hand washing as stated by the WHO. But only 24 per cent of the ICU staff actually followed all five moments of hand hygiene. These practices were almost similar to Mikatti et al<sup>(21)</sup>, which showed that although staff were well aware of proper hygienic practices, their performance was below the accepted recommendations. The modern NICU nursing concepts promoted minimal-handling protocol and clustering of nursing care. Study by Pittet et al showed that high demand for hand hygiene combined with high workload were the most significant risk factors for noncompliance<sup>(22)</sup>.

Our study showed that 85.7% of the staff had knowledge in use of personal protective equipments and fresh gloves. While, only 67.8% wore in practice. This was in line with the findings of Kennedy et al<sup>(5)</sup> and Sharma et al<sup>(20)</sup>, where healthcare workers surveyed also demonstrated high knowledge of in the usefulness of gloves to prevent the spread of infection, but the reported actual use of gloves was low. Gloves and PPE are equally essential to protect health care workers and patient too from cross infection through reducing the risk of exposing skin or mucous membranes to potentially infectious materials such as blood and other body fluids.

The present study had also established a link between patient's oral hygiene and infection control. About 53.6% of studied group satisfying knowledge about oral health care practices protocol, but only 35.7% actually did it in practice. The results were similar to Sharma et al<sup>(20)</sup> demonstrated that 80% of the staff had knowledge about patient's oral health care practices, 44% of the staff followed it as per the protocols.

In this study, a disconnect existed between central venous catheter care knowledge and practice. Although participants demonstrated high knowledge of appropriate CVC care (75%), the reported actual practice of such procedures as per protocol was low (57.1%). These results were in agreement with the findings of many studies conducted by Kennedy et al and Sharma et al<sup>(5,20)</sup>.

As revealed from the current study, about two thirds of the studied sample (64.3%) were aware how to dispose wastes and sharps off in a right way, only 46.3% frequently dispose them off in practice right way. That is in line with other similar studies conducted by Sharma et al<sup>(20)</sup>, Eskander et al<sup>(10)</sup>, and Kermodé et al<sup>(23)</sup>. In this regards, Schmid et al<sup>(24)</sup>, and Efstathiou et al<sup>(12)</sup> recommended that needles never should be recapped, as this could poses a serious danger through needle-stick injuries.

This study showed that 71.4% of HCWs had knowledge about instruments sterilization and 53.6% of them reported knowledge about proper environmental sanitation. While, only 64.3% frequently use sterilized instruments and only 28.6% of HCW follow environmental cleaning as recommended. On the same line with this finding was,

Ahmed et al<sup>(25)</sup> illustrated that nurses and laboratory technicians had unsatisfactory knowledge and practice. However, Alwutaib et al<sup>(14)</sup> reported acceptable knowledge level and practice about sterilization and environmental sanitation. Majority of NICU health care workers in our study were satisfied about instruments sterilization (85.7%), but only 39.3% of HCW reported that NICU environment is clean. Only 21.4% of the studied HCWs had received infection control training. The knowledge scores were significantly higher among HCWs who had received training on infection control than that among those who were not trained (91.7% and 63.1% respectively). Also, the practice scores were significantly higher among those who were trained on infection control than that among those who were not trained (76.2% and 39.6% respectively). This was in agreement with Kable et al<sup>(26)</sup> who found that just one-third of their studied nurses attended training courses about infection control, expressing more poor knowledge level and practices. However, Ebied<sup>(27)</sup> found that more than half of nurses attended infection control courses. Attending training programs increase the HCWs' knowledge and refine their practices which are helpful in preventing infection in ICU. So, training programs should be conducted in the hospital. This was also recommended by Eskander et al<sup>(10)</sup> and Sharma et al<sup>(20)</sup> who stressed on continuing nursing training programs especially in carrying out procedures that require strict aseptic techniques.

## CONCLUSION

There is inadequate overall knowledge level and poor compliance with standard precautions of infection control among NICU health-care staff in Libya especially among those who didn't receive training on infection control. A disconnect existed between knowledge and practice, inspite of having high knowledge about some IC standard precautions, their practices didn't reach satisfactory level.

## RECOMMENDATIONS

It is very important to develop infection control policy and protocols that strengthen the training of HCW in standard precautions to improve not only their knowledge but also their practice. Also, we recommend strict observation of HCWs' performance of infection control standard precautions to correct poor practices when required.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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# BURDEN OF HOSPITAL ACQUIRED INFECTIONS AND ANTIMICROBIAL USE IN LIBYA HOSPITALS ADULT INTENSIVE CARE UNITS

RNLNL  
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## ABSTRACT

Evaluate drug resistance of bacteria isolated from equipment placed close to patients in an Intensive Care Unit of a Central hospital in Gharian. Libya is a lower middle-income country with no national surveillance system for hospital-acquired infections (HAIs). We assessed the prevalence of hospital-acquired infections and antimicrobial use in adult intensive care units (ICUs) in Libya. This is a cross-sectional study. The samples were collected with swabs moistened with Trypticase Soy Broth, which were then cultured in sheep blood agar and MacConkey agar. The phenotypic identification performed was based on the morphology of the strains and biochemical results. The drugs resistance analysis was based on Kirby-Bauer's Disk Diffusion protocol. Results: A rate of 94.4% of the analyzed equipment was contaminated. The most frequently isolated microorganisms were: *Acinetobacter* sp., *Staphylococcus aureus* and *Pseudomonas* sp. Just about 75% of *Acinetobacter* sp. was resistant to piperacillin associated to tazobactam, meropenem and levofloxacin. Similarly, 36.3% of *S. aureus* showed resistance to oxacillin and 10% of *Pseudomonas* sp. was resistant to the drugs tested.

## INTRODUCTION

Healthcare-associated Infections (HAI) are responsible for thousands of deaths every year around the world. In Libya, this problem increases in terms of numbers as well as complexity, causing economic and social disruption with high levels of morbidity and mortality<sup>(1,2)</sup>. In Intensive Care Units (ICU), the contamination of equipment by bacteria is common, turning them into reservoirs of these microorganisms, enabling the colonization and cross infection of patients, complicating prognosis and favoring HAI outbreaks, mostly by microorganisms multiresistant to antibiotics commonly applied in therapeutics<sup>(3)</sup>, which implies severe limitations to the treatment of hospital infections, posing a great threat to public health<sup>(4)</sup>.

Different organisms are related to contaminations in hospital environments and HAI processes<sup>(5)</sup>, but the main pathogens include oxacillin-resistant *Staphylococcus aureus* (ORSA), vancomycin-resistant *Enterococcus* sp. (VRE) and, more recently, extended-spectrum beta-lactamases (ESBL) and carbapenem-resistant *Acinetobacter baumannii*<sup>(6-9)</sup>. Bacterial resistance is natural and unavoidable<sup>(3)</sup>, but the frequent and undistinguished use of antimicrobials (mainly broad-spectrum drugs) are crucial factors for the development and acceleration of this process<sup>(3,10)</sup>. Given these facts, the purpose of this study was to isolate and determine the drug resistance profile of bacteria isolated from ICU equipment in a hospital in Gharian.

## MATERIAL AND METHODS

This descriptive and cross-sectional study was developed in the Intensive Care Unit of a Central hospital located in Gharian, in the Aljabal region of Libya, from January to December 2014. Convenience sampling was applied, in which 54 pieces of equipment (right and left side rails and height adjustment buttons

from the beds, infusion pump buttons, individual light switches and cardiac monitor shelves) distributed among the nine beds present in the general ICU (Figure 1) were selected for collection. The inclusion criterion was samples from surfaces whose beds were occupied by their respective patients.



(Figure 1) Equipment collected (arrows) in each bed

The collected data were typed, validated and processed in the software Excel 2010 (Microsoft Office). Descriptive analysis was applied to obtain the percentage of samples.

The samples were collected six hours after the last time the hospital beds had been cleaned (corresponding to two hours after the end of the visiting period), so as not to interfere in the routine activities on site. Sterile swabs were used moistened with Trypticase Soy Broth (TSB) medium. Immediately after the collection, when the swabs were spun around their axis over the previously selected equipment, they were

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again stored in the medium and incubated at  $36 \pm 0.5^\circ\text{C}$  during 24 hours.

After the growth in TSB, the samples were seeded in sheep blood agar and MacConkey agar and also incubated at  $36 \pm 0.5^\circ\text{C}$  during 24 hours. Gram staining was performed, followed by the identification of the genera and/or species of the bacteria, according to macro and microscopic characteristics of the colonies and biochemical test results. For the identification of bacteria from the family *Enterobacteriaceae*, the carbohydrate fermentation test was used in Triple Sugar Iron (TSI), as well as biochemical tests using the Sulfide Indole Motility (SIM), Simmons' citrate and Christensen's Urea Agar growth mediums. Tests based on Oxidase and Polymyxin B were used for the identification of glucose-non-fermenting Gram-negative bacteria. The identification of *Staphylococcus* sp. was performed through catalase, DNase and Novobiocin tests. *Streptococcus* sp. were identified through

the characteristics of hemolytic activity, the use of Bile esculin agar, Brain Heart infusion (BHI) + NaCl 6.5% and optochin tests.

The drugs resistance analysis was based on Kirby-Bauer's Disk Diffusion protocol in Mueller-Hinton agar, as proposed by the Clinical and Laboratory Standard Institute<sup>(11)</sup>.

## RESULTS

It was observed that 94.4% of the analyzed equipment was contaminated by one or more bacterial species. The most numerous isolated bacteria were *Acinetobacter* sp., *Staphylococcus aureus*, Coagulase-negative *Staphylococci* (CoNS), *Staphylococcus saprophyticus*, *Enterococcus* sp., *Klebsiella pneumonia* and *Streptococcus viridans*, as presented in (table 1). Gram-positive bacilli were found in 33.3% of the beds.

(Table 1) Distribution of bacteria isolated on equipment

Microorganism	S1 n (%)	S2 n (%)	S3 a (%)	S4 n (%)	S5 n (%)	S6 n (%)	Total n (%)
Acinetobacter sp.	03(18.7)	04(25)	03(18.7)	01(6.25)	01(6.25)	04(25)	16(26.57)
S. aureus	04(36.3)	02(18.1)	01(9)	01(8)	02(18.1)	01(9)	11(19.64)
Pseudomonas sp	01(10)	01(10)	02(20)	01(7.01)	01(10)	04(40)	10(17.85)
S coagulase negative	0(0)	0(0)	01 (14.2)	03(42.8)	02(28.5)	01(14.2)	07(12.5)
S. saprophyticus	0(0)	02(28.5)	03(42.8)	0(0)	01(14.2)	01(14.2)	07(12.5)
Enterococcus sp	0(0)	0(3)	0(0)	01(33.31)	01(33.3)	01(33.3)	33(53.5)
klebsiella pneumonia	0(0)	0(0)	0(0)	0(0)	0(0)	01(100)	01(1.76)
S. viridians	0(0)	0(0)	0(0)	0(0)	0(0)	01(100)	01(1.78)
<b>Total Isolated</b>	<b>08(14.2)</b>	<b>08(16)</b>	<b>10(17.8)</b>	<b>07(12.5)</b>	<b>08(14.2)</b>	<b>14(25)</b>	<b>56(100)</b>

S1-right rail; S2-left rail; S3 - height adjustment buttons from the beds; S4 - Infusion pump buttons; S5 - individual illumination switches; S6 - cardiac monitors' shelf; N - number of isolated bacteria; %-percentage of isolated bacteria.

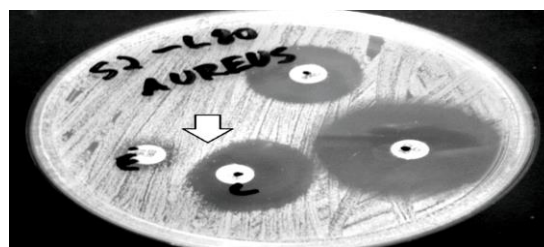
Among the *Acinetobacter* sp. isolated, 75% were resistant to imipenem, levofloxacin and piperacillin associated with tazobactam. 37.5% of the isolated microorganisms from this genus were resistant to ticarcillin, 31.2% to amikacin, 18.7% to ciprofloxacin, tetracycline and ceftazidime and 12.5% to gentamicin. 6.25% of the isolated microorganisms were found to have an intermediate level of resistance to ceftazidime and 12.5% to tobramycin.

With regard to the isolated *Staphylococcus aureus*, 72.7% were resistant to erythromycin, 63.6% to penicillin, 54.5% to clindamycin and ciprofloxacin and 18.8% to gentamicin. None of the isolated *Staphylococcus aureus* turned out to be resistant to ceftazidime. 9% of them had an intermediate level of resistance to oxacillin, clindamycin, erythromycin and ciprofloxacin. 36.3% were resistant to oxacillin (ORSA).

Among the Coagulase-negative *Staphylococci* strains, 71.4% and 54.1% were resistant to erythromycin and clindamycin, respectively. All these strains were susceptible to gentamicin, with 14.2% of them having the intermediate resistance phenotype to clindamycin. 42.8% were resistant to penicillin and 14.2% to tetracycline and ceftazidime.

The biggest number of cases of resistance to oxacillin in this study occurred in the isolated strains of *Staphylococcus saprophyticus*, which reached resistance levels in 85.7% of the samples, followed by 71.4% of resistance to erythromycin and clindamycin, 42.8% resistant to ciprofloxacin, 42.6% to ceftazidime, 28.5% to tetracycline and 14.2% to penicillin.

As regards the mechanism of inducible resistance to clindamycin, 12% of the *Staphylococcus* sp. had the positive phenotype. This was detected through the disk approximation test with erythromycin and clindamycin (Figure 2), in which 66.6% corresponded to Coagulase-negative S. and 33.3% to *S. aureus*.



(Figure 2) Inducible clindamycin resistance phenotype in *S. aureus*. E - erythromycin; C - clindamycin

Only 5.35% of the isolated strains corresponded to *Enterococcus* sp., 33.3% of which had intermediate levels of resistance to penicillin and ampicillin. None of the isolated strains were resistant to vancomycin.

A rate of 17.85% of the isolated strains corresponded to *Pseudomonas* sp., none of which turned out to have a significant level of resistance to the tested antibiotics (gentamicin, levofloxacin, aztreonam, ceftazidime, tobramycin, amikacin, ciprofloxacin, meropenem, chloramphenicol, cefoxitin and ticarcillin + clavulanic acid), while 10% of them were resistant and 20% presented intermediate levels of resistance to piperacillin + tazobactam; and 10% also had intermediate levels of resistance to ticarcillin + clavulanic acid and aztreonam.

Only one specimen of bacteria from the *Enterobacteriaceae* family (*Klebsiella pneumoniae*) was isolated in the study performed. Resistance was detected to ciprofloxacin, tetracycline, ampicillin, chloramphenicol and gentamicin, intermediate resistance to piperacillin + tazobactam and susceptibility to meropenem and tobramycin, and a negative result was obtained for Extended-Spectrum Beta-Lactamase (ESBL) production.

#### DISCUSSION

Part of the results reported in this study (be it the occurrence of genera and/or species of bacteria or their antimicrobial resistance profiles) corroborate what is described in the scientific literature, although comparisons sometimes tend to be inaccurate, since the sampling and microbial detection methods vary considerably among different studies<sup>(2)</sup>.

The high number of *Acinetobacter* sp. Isolated may have been due to its high level of nutritional and metabolic versatility, which allows this genus to use a large variety of substrates as carbon sources, remaining active for days or weeks in hospital environments<sup>(12,13)</sup>, which highlights the importance of their detection in such places, as this microorganism is directly involved in various ICU-related clinical complications<sup>(2)</sup>, and also in mechanisms of acquired resistance to carbapenem antibiotics<sup>(4)</sup>. Despite the existence of studies<sup>(2,4,6,13)</sup> on the genus *Acinetobacter* as an HAI agent from clinical samples, there is a lack of statistical data on the prevalence of this bacterium on hospital equipment. Besides that, the occurrence<sup>(4,13,14)</sup> of *Acinetobacter* sp. with multiresistant profiles is also considerable, which shows the need for more complex studies to determine the behavior of these strains in this kind of environment.

As regards the resistance profile of *S. aureus* strains to oxacillin (ORSA), a low number of cases was detected compared to the number of Gram-positives, although the result turned out to be superior when compared to a similar study, in which only 11.8% of the isolated strains had this resistance profile<sup>(15)</sup>. When the resistance of *S. aureus* strains to cefoxitin is analyzed, the results described in this study were similar to those of a study performed with biological

materials, in which no strains with this resistance profile were reported<sup>(16)</sup>.

As for the Coagulase-negative *Staphylococci* strains, no studies on their resistance profile in ICU equipment were found, but a study from 2006<sup>(17)</sup> reported lower resistance levels to erythromycin and clindamycin in biological samples: 68.7% and 63% respectively.

The occurrence of *S. saprophyticus* described in this study was superior when compared to studies from Libya, performed in 2014, in which only 3.3% of the analyzed pieces of hospital equipment were contaminated with this bacterial species<sup>(18)</sup>. Although the authors did not publish data on the antimicrobial resistance profile of *S. saprophyticus*, the levels for the genus *Staphylococcus* corresponded to 71.4% and 38.1% for erythromycin and ciprofloxacin, respectively<sup>(18)</sup>, corroborating the importance of their detection.

Despite the small number of *Staphylococcus* sp. strains with positive phenotype to induced resistance to clindamycin, this finding ought to be considered with special attention since the environment in question is one in which the patients are immunocompromised. Thus, the small number of occurrences must not be underestimated, as these bacteria have a great potential to cause hospital infections<sup>(2,3,5)</sup>. The low occurrence of vancomycin-resistant *Enterococcus* sp. strains differs from a study performed in the US using biological samples<sup>(19)</sup>, in which the estimated rate of resistance to this drug in ICU was of 17.7%. In Brazil, the first report of this phenotype dates back to 1998 and, in Latin America, the rise in the number of cases of this kind of resistance happened in countries like Chile, Uruguay and Argentina<sup>(20,21)</sup>.

The presence of *Pseudomonas* sp. strains as reported in this study corroborates what is reported in similar studies<sup>(22-24)</sup>, based on the analysis of equipment from different hospital environments, including ICU. In a hospital environment, the biggest sources of contamination from this microorganism are breathing equipment, hemodialysis systems, sinks and cleaning apparatus. The relevance of *Pseudomonas* sp. As a potential hospital pathogen depends on the bacterial species and is associated to its relative resistance to the drugs, as well as to its reduced susceptibility to the antiseptics and disinfectants used in these environments<sup>(25,26)</sup>. The drugs resistance profile of *Pseudomonas* sp. here described corroborates previous studies performed with biological material<sup>(27)</sup>, in which the major part of the antibiotics tested on *Pseudomonas aeruginosa* turned out to be effective against most of the isolated strains. The presence of these strains with mutually similar drug resistance profiles suggests the dissemination of a clone in the hospital environment; a fact that is probably related to cross-contamination mechanisms, although more extensive studies would be needed to confirm its dispersion in the ICU environment.

With regard to the percentage of isolated strains belonging to the *Enterobacteriaceae* family, the results obtained in this study differ from what is reported in studies performed in equipment from hospital environments<sup>(28)</sup>, where 30.3% were found to be contaminated by strains of this family. On the other hand, the antimicrobial resistance analysis of these strains in this study corroborates what is reported in the scientific literature: the massive presence of resistance to aminoglycosides and third-generation cephalosporins<sup>(7,29)</sup>.

When ESBL-production is considered, the results described in this study differ from those reported by Weber *et al.*<sup>(30)</sup> and Judge *et al.*<sup>(28)</sup>, in which a total of 22.2% of *Enterobacteriaceae* strains isolated on equipment from ICUs beds were ESBL-producing.

### CONCLUSIONS

High prevalence of HAIs in Libyan ICUs, mainly caused by Gram-negative bacteria with high levels of antimicrobial use illustrate the urgent need for capacity strengthening in both rational antimicrobial use and infection control efforts at national, regional and local levels. Most of the isolated strains had high rates of antimicrobial resistance to the drugs, which poses a great threat to public health

### SUPPORTING INFORMATION

Hospital and ICU Characteristics, Patient Characteristics, HAI Prevalence per Month, Antimicrobials Combinations Used. The Common Antimicrobial Agents Used.

### ACKNOWLEDGEMENT

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# ASSESSMENT OF MEDICAL WASTEWATER DISCHARGE FROM HOSPITALS IN LIBYA CASE STUDY: HOSPITALS IN EAST PART OF LIBYA

RNLNL  
139/2018

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## ABSTRACT

Hospital waste management is a crucial environmental and public safety issue. Discharge water is one of the main sources of groundwater and rivers contamination if not treated efficiently Hospitals consume an important volume of water a day, and generate multiple amounts of infectious and hazardous polluted discharge water to the drain. These pollutants should be treated by the wastewater treatment plant (WWTP) of each hospital before release to the municipal drainage. This study aims at presenting the primary results on characterization of hospital wastewaters in some hospitals in Northeastern part of Libya. Four major hospitals were selected for this study ranging from 500 to 120 beds per hospital, none of them have a wastewater treatment plant, and the wastewater is rejected untreated to the public sewage. This addition of polluted wastewater will increase the load to the WWTPs of the city and reduce the efficiency of treatment processes leading to hazardous pollution in the future. Analysis of the concentration of biochemical oxygen demand, chemical oxygen demand, total suspended solids, sulfate, nitrate, nitrite, ammonia, chlorides, and oils and grease. Heavy metals were also investigated as Cr, Cd, Pb, Hg, Cu, Ni and Zn. The bacteriological parameters were carried out using the standard microbiological techniques. The study was conducted during the period of (15/4/2014 - 15/6/2014). The study showed an increase in the concentration of pollutants which is higher than the Libyan and WHO guidelines of raw wastewaters.

**KEYWORDS:** Evaluation, Wastewater, Discharge, Hospitals, Libya.

## INTRODUCTION

In the last years, increasing attention has been paid to the presence of emerging pollutants in surface and groundwater such as surfactants and pharmaceuticals that their sales are continuously increasing<sup>(1,2)</sup>. Then, improvement of the hospital waste management must receive increasing attention where hospitals generate a considerable amount of medical waste each year as a result of advance in medical services and products<sup>(3)</sup>.

Hospitals produce relatively large quantities of wastewater that may contain various potentially hazardous materials; therefore, the proper management of hospital wastewater is vital. Hospitals wastewater has similar quality to municipal wastewater and important sources of pharmaceuticals residues in all wastewater treatment plant (WWTP) effluents due to their inefficient removal in the conventional systems<sup>(4)</sup> Moreover, Hospital wastewater includes a great variety of micro-contaminants that are chemicals, heavy metals, disinfectants and specific detergents resulting from diagnosis, laboratory, research activities and medicine excretion by patients. Indeed, hospital wastewater may have an adverse impact on environmental and human health; therefore, the proper management of hospital wastewater quality and quantity is needed.

The main objective of this study was to evaluate of wastewater discharge from hospitals in Northeastern Part of Libya to present the primary results of both biological and chemical characterization of the hos-

pital wastewater and identifying some environmental stressors released by hospital activities. Indeed, the study of wastewater quality and quantity in the investigated governmental hospitals to determine the suitable method for wastewater management quality in the hospitals environment for risk assessment as well as risk management related to hospital effluents.

## MATERIALS AND METHODS

The wastewater samples were collected from four wastewater outlets from different units of the four hospitals of the study area. Biochemical Oxygen Demand (BOD<sub>5</sub>), Chemical Oxygen Demand (COD), pH and Total Suspended Solids (TSS) were tested for raw wastewater samples. pH was measured on-site. Total heavy metals (Cr, Cd, Pb, Hg, Cu, Ni and Zn) were determined according to "Standard Methods for the Examination of Water and Wastewater"<sup>(5)</sup>.

The bacteriological parameters monitored include total viable aerobic counts to isolate heterotrophic bacterial and fungal, total coliform counts, Escherichia coli, faecal streptococci counts and Clostridium perfringens counts according to the methods of Gerhardt et al.,<sup>(6)</sup>. The isolating and identification of bacterial isolates were carried out according to Bergey's Manual of Determinative Bacteriology<sup>(7)</sup> The physiochemical parameters were studied using the Standard Methods of APHA<sup>(5)</sup>.

These Parameters are considered as significant factors by Libyan Environmental General Authority (LEGA) Libya, for monitoring of hospital wastewater disposal<sup>(8)</sup>.

Sample analysis was done according to the standard methods<sup>(5)</sup>. All of the analyses were conducted in the

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### RESULTS

The pH, TSS, BOD<sub>5</sub> and COD values are illustrated in (table 1) and (figure 1-a) show the average pH value of wastewater in the investigated hospitals. The average TSS concentration of wastewater in the studied hospitals is illustrated in (table 1), and (figure 1-b). The average BOD<sub>5</sub> and COD concentrations of wastewater in the investigated hospitals are represented in (table 1), and (figure 1-c). The aver-

age COD to BOD<sub>5</sub> ratio (COD/BOD<sub>5</sub>) of wastewater in the studied hospitals is illustrated in (figure 1-d). In the current study, Total coliform TC was indicator of the presence of microorganisms. Maximum and minimum concentrations of TC in wastewater of the studied hospitals were  $1.9 \times 10^9$  and  $2.5 \times 10^3$  MPN/100 mL.

In (table 2). The concentration of heavy metals such as (Cr, Cd, Pb, Hg, Cu, Ni and Zn) in the investigated hospitals effluent is presented.

(Table 1) The characteristics of raw wastewater in studied hospitals

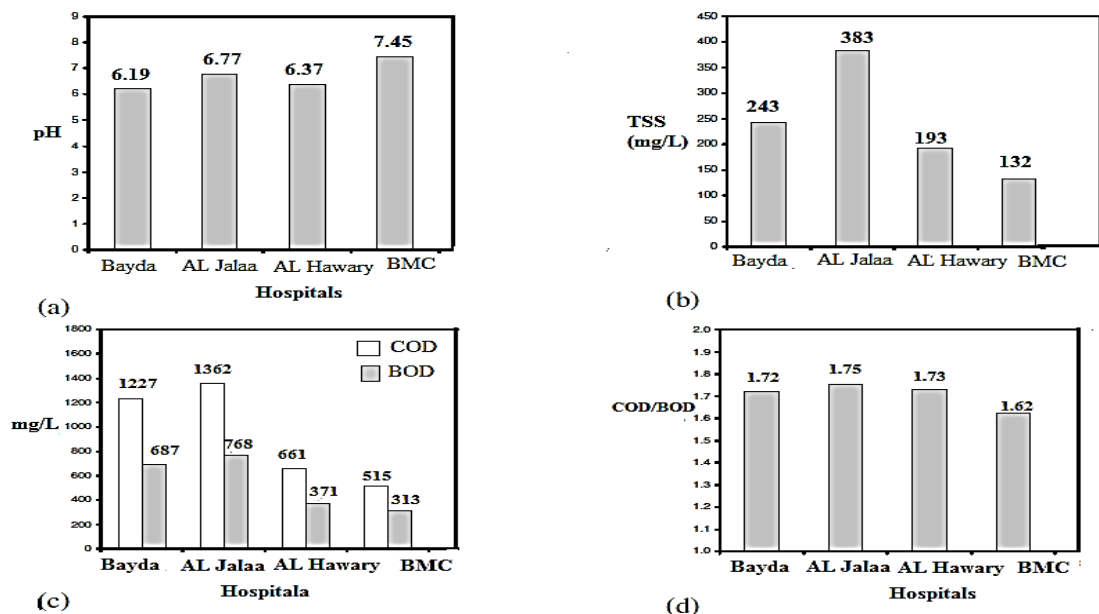
Hospital Name	pH	TSS (mg/L)	BOD <sub>5</sub> (mg/L)	COD (mg/L)	Total Coliform TC MPN/100 mL
Benghazi Medical Center (BMC)	7.45	132	313	515	$1.9 \times 10^9$
AL Jalaa Benghazi	6.77	383	768	1362	$3.3 \times 10^8$
AL Hawary Hospital	6.37	193	371	661	$4.1 \times 10^6$
AL Bayda Hospital	6.19	243	687	1227	$2.5 \times 10^3$
Mean	6.7	237.75	534.80	941.30	$2.23 \times 10^9$
<sup>†</sup> Libyan Environmental General Authority (EGA) Guidelines	6 - 9	600	500	1000	$1.0 \times 10^3$

<sup>†</sup>EGA guidelines for raw wastewater to the public network

(Table 2) Heavy Metals Concentration of Study Hospitals Wastewater

Hospital Name	Cr $\mu\text{g/L}$	Cd $\mu\text{g/L}$	Pb $\mu\text{g/L}$	Hg $\mu\text{g/L}$	Cu $\mu\text{g/L}$	Ni $\mu\text{g/L}$	Zn $\mu\text{g/L}$
Benghazi Medical Center (BMC)	44.5	3.93	44	14.4	59	33.6	469
AL Jalaa Benghazi	38.8	2.62	36	10.5	48.6	30.4	447
AL Hawary Hospital	39.6	2.10	24	7.6	51.2	27.5	427
AL Bayda Hospital	30.7	1.76	18	4.1	31.7	23.8	398
Mean	38.4	2.85	30.5	9.15	47.63	28.83	435.3
<sup>†</sup> Libyan Environmental General Authority (EGA) Guidelines	1200	20	1000	50	1200	2000	2600

<sup>†</sup>EGA guidelines for raw wastewater to the public network



(Figure 1) Quality characteristics of wastewater in the investigated hospitals;

(a) pH, (b) TSS, (c) COD and BOD<sub>5</sub> and (d) COD/BOD<sub>5</sub> ratio.

### DISCUSSION

The acidic or basic wastewater damages the wastewater collection and treatment facilities and prevents the biological treatment processes.<sup>(9)</sup> According to (figure 1-a), the minimum and maximum pH values were observed in the wastewater of AL

Bayda Hospital and Benghazi Medical Center (BMC), so the average pH value of wastewater in investigated hospitals was obtained to be 6.7. The mentioned range of pH (9–6) is suitable from the viewpoint of wastewater treatment processes and comparable to pH of domestic wastewater<sup>(10)</sup>. The

equivalent results were obtained in the other studies. Clean Technology Consultant (1994)<sup>(9)</sup> determined the wastewater pH value of a hospital in Thailand to be 7.2. In Indonesia, the range of pH in the hospital wastewater was obtained in range of (5.9 – 12.5)<sup>(6)</sup>.

One of the common parameters used in defining a wastewater is total suspended solids (TSS). According to (figure 1-b), the minimum and maximum TSS concentrations were obtained in the wastewater of Benghazi Medical Center (BMC) and AL Jalaa Benghazi Hospital, so the average TSS concentration of wastewater in the investigated hospitals was obtained 237.75 mg/L. The average TSS concentration of domestic wastewater is in the range of (120-600 mg/L) that is comparable to the results obtained in the investigation<sup>(8)</sup>.

The parameters of BOD<sub>5</sub> and COD are widely used to characterize the organic matter content of wastewater. According to Figure (1c), the minimum and maximum concentrations of BOD<sub>5</sub> were obtained in Benghazi Medical Center (BMC) and AL Jalaa Benghazi Hospital, so the average concentrations of BOD<sub>5</sub> were determined to be 313 and 768 mg/L, respectively. According to (figure 1-c), the minimum and maximum concentrations of COD were obtained in Benghazi Medical Center (BMC) and AL Bayda Hospital, so the average concentrations of COD were determined to be 515 and 1227 mg/L respectively. the most of the hospitals, the BOD<sub>5</sub> and COD concentrations of wastewater are almost equal to the domestic wastewater values<sup>(9)</sup>, determined the BOD<sub>5</sub> and COD concentrations of a hospital wastewater to be 113 and 232 mg/L, respectively. In Bangkok the BOD<sub>5</sub> and COD concentrations of a hospital wastewater were 300 and 430 mg/L, respectively<sup>(10)</sup>. According to (figure 1-d), the COD/BOD<sub>5</sub> ratio was in the range of (1.62-1.75), however in the domestic wastewater, the ratio is around 2.3. Therefore, the organic matters in the hospital wastewater had higher biodegradability in comparison with domestic wastewater. The high biodegradable of organic matters is very desirable from the viewpoint of wastewater treatment and promotes the efficiency of wastewater treatment plants<sup>(11)</sup>.

A serious concern regarding wastewater is the high content of enteric pathogens including bacteria, viruses, protozoa and helminthes, which are easily transmitted through water<sup>(12)</sup>. Wastewater of hospitals where patients with enteric diseases are hospitalized is a particular problem during outbreaks of diarrheal diseases<sup>(13)</sup>. In the present study, total coliforms (TC) were selected as indicator of the presence of microorganisms. According to (table 1), the minimum and maximum numbers of total coliforms TC were obtained in the wastewater effluents of AL Bayda Hospital and Benghazi Medical Center (BMC), so the average number of total coliforms in the investigated hospitals wastewater was obtained to be  $2.23 \times 10^9$  MPN/100 mL. The average number

of total coliforms in domestic wastewater is in the range of 106-10<sup>10</sup> MPN/100 mL that is comparable to the results obtained in the investigation.

The source of heavy metal in hospital wastewater can be originated from numerous sources such as from feces, cleaners, paints and wear and tear of utensils and equipment<sup>(2)</sup>. Certain heavy metals are micronutrients essential for plant growth (e.g., copper and zinc) and provide benefit to the crop. However, they can present problems for plant growth at excessive amount. Some are also not essential plant or animal and toxic to animals and human such as arsenic, cadmium and mercury. Heavy metals are a major concern in the treatment of water and wastewater due to their toxic and other detrimental effects.

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# FUNGAL AND YEAST EVALUATION OF YOGHURT IN MISURATA CITY, LIBYA

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## ABSTRACT

Yoghurt is one of the most well-known food products that contain a variety of microbes that has the ability to render the customers into danger. The aim of this study is to evaluate the microbial content of fermented milk products in Misurata's markets, 20 samples have been collected for plain, fruit, and flavored yoghurt. These samples were transported to the lab to perform microbial examinations. First of all, the pH was measured in the yoghurt samples which were  $4.58 \pm 0.27$  in plain yoghurt,  $4.6 \pm 0.23$  in fruit yoghurt and  $4.61 \pm 0.22$  in flavored yoghurt. In addition to bacteria, some yeasts and fungal species isolated including *Saccharomyces cerevisiae*, *Candida parapsilosis*, *Pichia*, *Rhodotorula rubra*, *Penicillium*, *Aspergillus*, and *Rhizopus* were isolated from the examined plain yoghurt samples in the percentage of 50%, 37.5% and 12.5% respectively. While in fruit yoghurt only *Mucor* and *Penicillium* were isolated giving 33.3, 66.6% respectively, while in flavored yoghurt only *Penicillium* was isolated by the percentage 100%. When our results were compared with the Libyan standard 4 out of 20 samples were not acceptable in all types of examined yoghurt.

**KEYWORDS:** Yeast, Fungi, yoghurt.

## INTRODUCTION

The generic name of fermented milk was derived from the fact that the milk destined for the product is inoculated with a starter culture which converts part of Lactose into Lactic acid. Carbon dioxide, Acetic acid, Diacetyl acetaldehyde and several other substances are formed in the conversion process, and these impart to the products their characteristic fresh taste and aroma<sup>(7)</sup>. Fermented milk helps to maintain food and water intakes which are often reduced in elder people. Beyond their good taste, fermented milk and especially yoghurts have unique nutritional attributes. These foods contain high levels of proteins, calcium, phosphorus and vitamins B<sub>2</sub> and B<sub>12</sub><sup>(18,20)</sup>. The digestive system of some people lacks of the lactase enzyme. As a result of that, the Lactose will not be broken down in the digestive process into simpler types of sugars and those people cannot drink ordinary milk. On the other hand, they can eat cultured milk in which the lactose is already partially broken down by the bacterial enzymes<sup>(17)</sup>. Yeasts and fungi can enter foods through inadequately sanitized equipment or as air-borne contaminants, moreover, can tolerate the psychrotrophic temperature, therefore they become a problem in dairy products<sup>(21)</sup>.

## MATERIALS AND METHODS

### Collection of samples:

A total of 60 random samples of plain yoghurt, fruit yoghurt and flavored yoghurt, (20 for each) collected from different localities in Misurata city, from January 2017 to March 2017.

### Preparation of samples:

On arrival to the laboratory each sample was perfectly mixed before being divided into two

sub-samples. The first one used for determination of pH, while the second was examined bacteriologically.

### Determination of pH:

Standard method<sup>(4)</sup> was used to determine pH value by using pH meter (Jenway- model:3505, Made in UK).

### Yeast and fungi count<sup>(4)</sup>:

One ml from the previously prepared dilutions was inoculated into duplicate plates. Then about 15 ml sterile Sabaroud Dextrose agar cooled to 45°C, were poured into each petri-dish. Inoculated plates, after being mixed and solidified, were incubated at room temperature (25°C) for 5 days, then yeast and fungi count ml was calculated.

### Identification of isolated fungi:

The incubated plates were examined visually and microscopically. The individual colonies of fungal isolates were selected depending upon their morphological characters and microscopical examination. Members of the Genus *Aspergillus* were classified according to the keys of<sup>(19)</sup>.

Stock cultures were made from the isolates and examined on Saboroud Dextrose agar slopes for further identification. Identification of the colonies were carried out by careful observation of the macroscopic and microscopic characteristics of the fungal colonies as follow:

### Macroscopic examination:

This depends on the observation of the rate growth of colonies, texture and pigmentation on the surface and reverse side over a period of one week.

### Microscopic examination:

#### Direct microscopic examination:

From 4-6 days old colonies, a piece was transferred to a clean glass slide, and then one drop



of lactophenol cotton blue was added. The slide was covered with a glass cover and examined microscopically.

**Identification of isolated yeasts:**

Examination for morphological characters of the isolated yeast facilitated the identification and classification of them into genera.

**Gross appearance of yeast colonies:**

The colonies characteristics were described considering the rate and pattern of growth, its size, texture and surface color.

**Direct microscopic appearance:**

Carried out by placing a part of the colony on a slide with one drop of distilled water, then cover with cover slip and examined under 100X of magnification power for the presence of yeast cells.

**Biochemical properties of yeast:**

For identification of yeast into species, biochemical tests have been done.

**1- Sugar fermentation<sup>(6)</sup>:**

Media containing sugar were inoculated with the isolates suspension and incubated at 25°C for 3 days.

Positive case indicated by production of acid (medium turn to yellow color).

**2- Sugar assimilation test<sup>(23)</sup>:**

Sugar assimilation medium was cooled at 45°C poured into sterilized petri dishes and mixed with 0.2ml saline suspension of the isolates. The plates were left to dry at room temperature. Filter paper discs soaked in 20% solution of different sugars were placed on the surface of the media, and then incubated for 5 days at 25°C. Presence of growth around the disc indicated the utilization of sugars.

**Statistical analysis:** Done by ANOVA one way and t- test

**RESULTS AND DISCUSSION**

The results described in (table 1) show that the pH in examined plain yoghurt samples was ranged from 4.2 to 5.1 with a mean value of 4.58±0.27 and in examined fruit yoghurt samples was ranged from 4.2 to 5.1 with a mean value of 4.61±0.23. while in examined flavored yoghurt, samples were in a range from 4.3 to 5.0 with a mean value of 4.61±0.22. Nearly similar data were obtained by<sup>(14,15)</sup>.

**(Table 1)** Statistical analytical results of pH in examined yoghurt samples.

Product	No.	Min.	Max.	Mean ± S. D	p. value
Plain	20	4.2	5.1	4.58 ± 0.27	0.902
Fruit	20	4.2	5.1	4.61 ± 0.23	
Flavored	20	4.3	5.0	4.61 ± 0.22	

A careful inspection of (table 2) shows that according to frequency distribution of examined yoghurt samples based on pH, the highest frequency distribution of the examined plain yoghurt samples (25%) lies within the range of

4.31-4.4, and the highest frequency distribution of examined fruit yoghurt samples (20%) lies within the range of 4.3-4.4 and 4.4-4.5 while (25%) of examined flavored yogurt samples were lies within the range 4.5-4.6.

**(Table 2)** Frequency distribution of examined yoghurt samples based on their pH.

interval	Plain yoghurt		Fruit yoghurt		Flavored yoghurt	
	No.	%	No.	%	No.	%
4.21-4.3	4	20%	2	10%	3	15%
4.31-4.4	5	25%	4	20%	2	10%
4.41-4.5	1	5%	4	20%	2	10%
4.51-4.6	3	15%	2	10%	5	25%
4.61-4.7	2	10%	2	10%	3	15%
4.71-4.8	1	5%	3	15%	1	5%
4.81-4.9	1	5%	1	5%	3	15%
4.91-5.0	2	10%	1	5%	1	5%
5.01-5.1	1	5%	1	5%	0	0%
Total	20	100%	20	100%	20	100%

(Table 3) demonstrate that the total yeast and fungi count in examined plain yoghurt samples was ranged from 0.1X10 to 1.2X10 with a mean value 0.48X10± \0.29X10 and in examined fruit yoghurt samples was ranged from 0.1X10 to 1.6X10 with a mean value 0.56X10±0.36X10. While it was ranged from 0.1X10 to 1.0X10 with a mean value 0.49X10±0.28X10 in examined flavored yoghurt samples. Nearly similar data were reported by (1, 2, 5, 11). While higher counts were obtained by (3, 10, 12, 16, 22, 24).

**Table (3)** Statistical analytical results of total yeast and fungi count/ml in examined yoghurt samples.

Examined yoghurt samples	No. of examined samples	Positive samples		Count/ml		Mean ± S. D	p. value
		No.	%	Min.	Max.		
Plain	20	20	100%	0.1X10	1.2X10	0.48X10± 0.29X10	0.979
Fruit	20	20	100%	0.1X10	1.6X10	0.56X10± 0.36X10	
Flavored	20	20	100%	0.1X10	1.0X10	0.49X10± 0.28X10	

The results present in (table 4) indicate that most of the examined plain yoghurt samples (95%), (90%) of examined fruit yoghurt samples and (95%) of examined flavored yoghurt samples based on their total yeast and fungi count lies within <10.

**(Table 4)** Frequency distribution of examined yoghurt samples based on their yeast and fungi count.

interval	Plain yoghurt		Fruit yoghurt		Flavored yoghurt	
	No.	%	No.	%	No.	%
10>	19	95%	18	90%	19	95%
10-10 <sup>2</sup>	1	5%	2	10%	1	5%
Total	20	100%	20	100%	20	100%

The results reported in (figure 1) show that *Saccharomyces cereviceae* was predominant yeast by 43.7%, *Pichia*, *Rhodotorula rubra*, *Candida parapsilosis*, and *candida albicans* were isolated from the contaminated examined plain yoghurt samples in the percentage of 18.7%, 18.7%, 12.5% and 6.2% respectively. While in fruit yoghurt *Saccharomyces cereviceae* was isolated in 38.4% of positive samples then *Candida parapsilosis*, *Pichia*, *Rhodotorula rubra*

and *Cryptococcus uniguttualtus* isolated by the percentage of 23.07%, 15.3%, 15.3% and 7.6% respectively. While in flavored yoghurt also *Saccharomyces cereviceae* was predominant yeast by 50% then *Candida parapsilosis* 21.4%, *Rhodotorula rubra* 14.2%, *Pichia* and *Cryptococcus uniguttualtus* were giving similar results (7.1%). *Candida albicans* was not isolated in fruit and flavored yoghurt samples. These data were nearly with<sup>(9,18)</sup>.

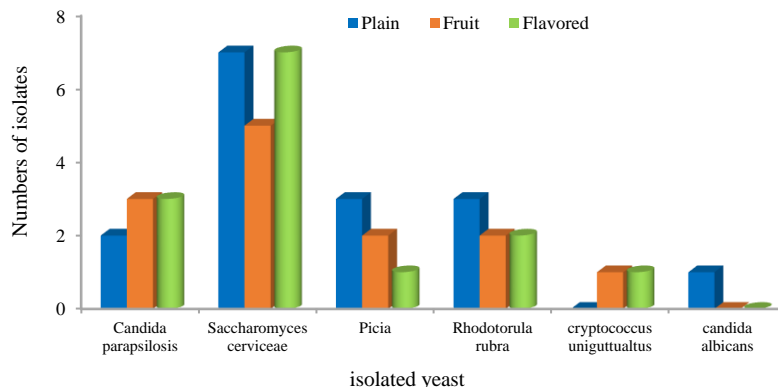


Figure (1) Incidence of isolated yeast in examined yoghurt samples.

The results reported in (figure 2) demonstrate that *Penicillium*, *Aspergillus*, and *Rhizopus* were isolated from the examined plain yoghurt samples in the percentage of 50%, 37.5% and 12.5% respectively. While in fruit yoghurt only *Penicillium* and *Mucor* were isolated by the percentage of 66.6% and 33.3% respectively. While in flavored yoghurt only *Penicillium* was isolated by the percentage 100%. These results were agreed with<sup>(8,12,13)</sup>.

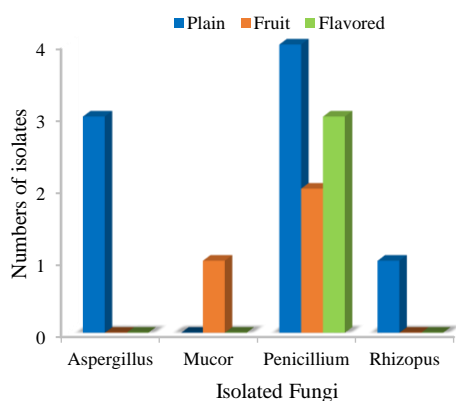


Figure (2) Incidence of isolated fungi in examined yoghurt samples.

(Table 5) reports that just 1 plain yoghurt samples unacceptable with the yeast count, while in examined fruit yoghurt samples there were 2 samples unaccepted with yeast count in compare with Libyan standard, and in flavored yoghurt samples most of the samples were going with the Libyan standard according to yeast count which were 19 accepted with the Libyan standard (2005\559).

(Table 5) Comparison between Libyan standard criteria and examined yoghurt samples.

Standard	Plain samples		Fruit samples		Flavored samples	
	Unacceptable	Acceptable	Unacceptable	Acceptable	Unacceptable	Acceptable
	No	%	No	%	No	%
Yeast	<1	5	2	10	1	5
	0	19	95	18	90	19
		%	%	%	%	%

**CONCLUSION AND RECOMMENDATIONS**

The information given by the results of microbiological examination allow to conclude that most of yoghurt in Misurata city accepted with Libyan standard criteria and satisfy the consumer’s demand in obtaining such products in good sanitary condition and retaining as far as possible their nutritive value. According to all of our findings and to save the consumer from being infected and to save a lot of products from being spoiled on the market, the following suggestions are recommended:

- A suitable sanitizer should be used to prepare animals and ensure that their udders are clean.
- Strict hygienic measures should be applied during milking, collection and transportation.
- Only healthy persons who have sanitary certificates should be employed and should pass periodical medical examination.

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# INHALATION INJURY IN BURN PATIENTS ADMITTED TO ICU IN BURN AND PLASTIC SURGERY HOSPITAL DURING 2015 AND 2016

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## ABSTRACT

Inhalation injury has now become the most frequent cause of death in burn patients. Although mortality from smoke inhalation alone is low (0–11%), smoke inhalation in combination with cutaneous burns is fatal in 30 to 90 percent of patients. It has been recently reported that the presence of inhalation injury increases burns mortality rate. Identify incidence and epidemiological features of inhalation injury among fire burned patients and analysis of the outcome of our management. Retrospective study of all patients admitted to ICU burn in Burn and Plastic Surgery Hospital, Tripoli-Libya with inhalation injury from January 2015 to December 2016. Total number of admitted patients to ICU was 293, out of them 161 (55%) sustained inhalation injury, 126 (78%) patients out of 161 patients were male gender, 138 (86%) patients out of 161 were more than 15 y old in age, 70% of non-survived patients group were with inhalation injury. High incidence of inhalation injury among patients with fire burn who admitted to ICU, Inhalation injury increase mortality by 20%, Inhalation injury had occurred more frequently in adult male gender, Bad prognosis in inhalational injured patients complicated by infection and septicemia, Early diagnosis and intubations decline mortality and morbidity rate.

**KEYWORDS:** Smoke inhalation, Burns, Early intubation.

## INTRODUCTION

In developing countries incidence of inhalational injury is increasing because of rise in fire disaster, and Mortality from pulmonary injury are increasing<sup>(1)</sup>. Diagnosis not always straight forward because of lacking of sensitive tests and symptoms may be delayed up to 24-36 hour post burn<sup>(1,2)</sup>.

The prognosis of fire victims usually is determined by many factors including extent and duration of smoke exposure, chemical composition of the smoke, size and depth of body surface burns, temperature of gases inhaled, age (prognosis worsens in the very young and old) and preexisting health status<sup>(3,4)</sup>.

## AIM OF THE STUDY

- 1- Identify incidence and epidemiological features of inhalation injury among fire burned patients
- 2- Analysis of outcome of our management

## METHOD AND MATERIALS

This research is retrospective study of all patients admitted to ICU burn in Burn and Plastic Surgery Hospital, Tripoli-Libya (which is the only specialized hospital in Libya for treating burn injury victims) with inhalation injury from January 2015 to December 2016, (Age, sex, burn surface, complication, management, length of stay and outcomes are included).

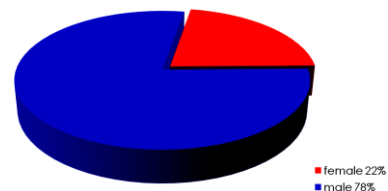
## RESULTS

- Total no. of admitted patients to ICU was 293, out of them 161 patients sustained inhalation injury. (table1)

**(Table 1)** Percentage of admitted pt. with inhalation injury

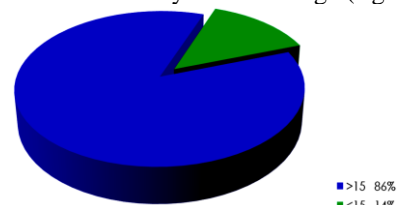
Total number of admitted patients. 293 (45%)	Total number of patients with inhalation injury. 161 (55%)
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- Male gender in Inhalation injury patients group was 126 patients that represents 78% (figure 1).



**(Figure 1)** Shows gender percentage among inhalation injured patients.

In Inhalation injury patients group, 138 (86%) out of them was more than 15 years old in age (figure2).



**(Figure 2)** Shows age distribution of admitted patients with inhalation injury.

- Total mortality rate in burn ICU was 100 patients (34%) (table 2).

**(Table 2)** Mortality rate among burn ICU patients.

Total number of admitted patients. 293	Total number of non-survived patients 100(34%)
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- Seventy percent of non-survived patients group were with inhalation injury (table3).

**(Table 3)** Shows percentage of non-survived patients with inhalation injury among non-survived patients in burn ICU.

Total no of non-survived patients 100 (100%)	Total no. of non-survived patients with inhalation injury 70 (70%)
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The mortality rate in the two groups of patients (with inhalation and without inhalation) was shown in (table 4).

**(Table 4)** Shows mortality rate in patient's groups with and without inhalation injury

Total number of patients without inhalation injury. 132. ↓↓↓	Total number of patients with inhalation injury. 161. ↓↓↓
Total number of non-survived patients without inhalation injury. 30 (23%)	Total number of non-survived patients with inhalation injury. 70 (43%)

The mean percentage of burn surface in non-survived patients. When associated with inhalation injury was lower than who have no inhalational injury (table 5).

**(Table 5)** Shows the mean percentage of burn surface in non-survived patients.

The mean percentage of burn surface of non-survived patients (without inhalation injury) ↓	The mean percentage of burn surface of non-survived patients (with inhalation injury) ↓
52% TBSA	40% TBSA

- In comparison between two groups, survived and non-survived of the patients with inhalational injury in terms of infection, time of intubation and length of stay were found, septicemia and pneumonia seen more frequently in non-survived group, also the time of intubation was delayed in this group, where the mean of length of stay in hospital was 45 days in survived group (table 6).

**(Table 6)** Shows comparison between 2 groups, survived and non-survived of the patients with inhalational injury.

Parameter	Survived		Non-survived	
	Female	Male	Female	Male
Sex	43%	57%	23%	77%
percentage	38 %	TBSA	40 %	TBSA
Septicemia	75 %		90 %	
pneumonia	48 %		65 %	
Time of intubation	1 <sup>st</sup> 24 hours		3 <sup>rd</sup> day	
Length of stay	45 days		9 days	

### DISCUSSION

High incidence of inhalation injury among the cases admitted to ICU burn because of fire accident which represent more than 50%<sup>(5,6)</sup>, that lead to high mortality rate (70%) of total expired cases<sup>(7)</sup>.

The main value of the burn surface in non-survived patients with inhalation in the present study was found less than those who had no inhalation injury supported the earlier studies that the inhalation injury was main prognostic factors<sup>(7,8,9)</sup>. The difference between the survived and non-survived male patients was found to

be more than 50% sustained that the inhalation injury was more in male patients<sup>(9)</sup> as reported earlier.

Inhalation injury complicated by septicemia and pneumonia which occurred more frequently in non-survived patients, which reveal bad prognosis in inhalational injury<sup>(10)</sup>.

The mean time of intubation in the survived patients was within 1<sup>st</sup> 24-hour post-burn and in non-survived patients were at 3<sup>rd</sup> day post – burn, maybe due to delay in symptoms and difficulty in diagnosis, so we consider the early intubation is live saving<sup>(11,12)</sup>.

In non-survived patients the mean duration of stay in ICU before death was nine days post burn (can be considered critical time for inhalation injured patient?) and in survived patient was 45 days.

Another factor that may be play important role in the prognosis of inhalation injury like extent and duration of smoke exposure, chemical composition of the smoke, temperature of gases inhaled, and preexisting health status of the patients that need more investigations.

### CONCLUSION

- Inhalation injury increase mortality by 20%<sup>(7)</sup>.
- The inhalation injury had occurred more frequently in adult male gender because of outdoor accident<sup>(7,8)</sup>.
- Septicemia and pneumonia are bad prognostic signs in inhalation injured patients.
- Early diagnosis of inhalational injury and early intubations of highly suspicious of inhalational injury in burned patients decline mortality & morbidity rate<sup>(11,12)</sup>.

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## HCV TRANSMISSION IN HEALTH CARE SETTING

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### ABSTRACT

An exposure that might place CHW at risk for HCV infection is defined as a percutaneous injury (e.g. a needle stick or cut with a sharp or contact with mucus membrane or non-intact skin or other body fluids that are potentially infectious) Feces, nasal secretions, saliva, sputum, sweat, tears, urine, vomitus, are not considered potentially infectious unless they contain blood. Avoiding occupational blood exposures is the primary way to prevent transmission of HCV in health care settings. HCV is not transmitted efficiently through occupational exposures to blood. The average incidence of anti HCV seroconversion after accidental percutaneous exposure from an HCV positive source is 1.8% (Range 0% -7%) Transmission occurred only from hollow –bore needle, compared with other sharps. Data are limited on survival of HCV in the environment. In contrast to the HBV the epidemiologic data for HCV suggest that environmental contamination with blood containing HCV is not a significant risk for transmission in the health care settings with the possible exception of the hemodialysis setting where HCV transmission related to environmental contamination and poor injection control practices have been implicated. The risk for transmission from exposure to fluids or tissues other than HCV infected blood also has not been quantified but is expected to be low. Data upon which to base a recommendation for therapy of acute infection are insufficient because no data exist regarding the effect of treating patients with acute infection who have no evidence of infection.

### INTRODUCTION

HCV is a single stranded RNA flavivirus. Six major HCV subtypes exist with varying amino-acid sequences (genotypes), these subtypes vary geographically and in virulence and response to therapy.

HCV can also alter its amino-acid pattern over time in an infected person.

Tattoos body piercing. Sexual transmission vertical transmission from mother to infant are relatively rare. Transmission through blood transfusion has become very rare since the advent screening tests for donated blood. Some in patients without apparent risk factors.

HCV prevalence varies with geography and other risk factors. HCV infection sometimes occur simultaneously with specific systemic disorders including mixed cryoglobulinemia, porphyria and glomerulonephritis, the mechanisms are uncertain.

In addition, up to 20% of patients with alcoholic liver disease harbor HCV. The reason for this high association are unclear because concomitant and drug use accounts for only a portion of cases. In these patients HCV and alcohol act synergistically to exacerbate liver damage.

### MATERIALS AND METHODS

The number of 182 reported cases of physicians, nurses and laboratory technicians working in 6 Regional hospitals in the Republic of Guyana South America were exposed to percutaneous injuries at work during the period from 1st January 2011 to 31st December 2012. Consent was obtained for both the exposure source and the exposed in accordance with applicable laws in the Republic of Guyana. For the source persons testing for anti-HCV testing was performed. Repeatedly reactive results by ELA were considered. 20 exposure source persons could not be tested. The exposure persons were evaluated for the potential transmission of HCV based on the type of

substance involved and the route and severity of the exposure. Blood was the main type of exposure through percutaneous injuries and other penetrating sharps were related to all events. For the exposed persons to an HCV- activity was performed. All exposure persons were followed up at 4-6 months, for anti HCV and ALT activity. All anti-HCV results reported positive were confirmed by enzyme immunoassay using supplemental anti HCV testing. (RIBA).

### RESULTS

A hundred eighty-two HPW were exposed to percutaneous and penetrating sharps. The average risk of transmission of Hepatitis C virus after needle stick was 1.8%. Liver enzymes (ie ALT) were abnormal in all exposure source persons. 50% of the exposed persons had drug use

Or sexual contact with a known positive partner.

### DISCUSSION

HCV transmission related to environmental contamination and poor injection control practices have been implicated. The risk for transmission from exposure to fluids or tissues other than HCV infected blood also has not been quantified but is expected to be low.

Data upon which to base a recommendation for therapy of acute infection are insufficient because no data exist regarding the effect of treating patients with acute infection who have no evidence of disease. The appropriate regimen is unknown.

### CONCLUSION

procedures should be established for testing CHW for HCV after percutaneous or mucosal exposures to blood and ensure that all personnel are familiar with these policies and procedures.

To confirm all anti-HCV results reported positive by enzyme Policies and Immunoassay.

Health care professionals who provide care to persons exposed to HCV in the occupational setting should be knowledgeable regarding the risk for HCV infection.

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