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Antibiogram Profiles of Bacterial Isolates from Intensive Care Units in Mosul Teaching Hospitals

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ABSTRACT

The study was conducted to identify the types and frequencies of bacterial isolates from patients with Intensive Care Units-acquired infections, and to determine their antibiogram profile. One hundred and fifty four clinical samples were collected from 101 patients who developed clinical suggestion of new infections of urinary tract, lower respiratory tract or wound following the 48 hours of their admission to the Intensive Care Units. All samples were cultured on different culture media, and the isolated microorganisms were identified by the conventional bacteriological methods. The antibiogram profile of selected antibacterial agents was tested. The total number of the bacterial isolates was 69 miroorganisms. Gram-negative bacteria were more frequently encountered (82.6%) than the Gram-positive ones(17.4%) among all samples. The predominant Gram-negative isolate was *E. coli* (23.2%), followed by *Pseudomonas* spp. (21.7%), *K. pneumoniae* (14.5%), *Proteus* spp. (11.6%)., *Acinetobacter* spp. (8.7%), and *E.aerogenes* (2.9%). The predominant Gram-positive bacterial isolate was *S.aureus* (7.2%), followed by *E. faecalis* (5.8%), and *S.epidermidis* (4.3%). The most effective antimicrobial agents were amikacin, ciprofloxacin, vancomycin, gentamicin, chloramphenicol, and azithromycin.

Keywords: ICUs, Bacterial isolates, Antibiogram profile.

أنماط التحسس للمضادات الحيوية للجراثيم المعزولة من وحدات العناية المركزة في مستشفيات الموصل التعليمية

الملخص

أجريت هذه الدراسة لتحديد أنواع و تكرار الجراثيم المعزولة من المرضى الذين يعانون من أخماج مكتسبة في وحدات العناية المركزة مع فحص حساسيتها للمضادات الحيوية. إذ تم جمع 154عينة من 101 مريض أشتبه بأن لديهم اخماج في الجهاز البولي او الجهاز التنفسي او الجروح بعد مرور 48 ساعة على رقودهم في وحدات العناية المركزة. زرعت العينات على أوساط زرعية مختلفة وتم تشخيصها باستخدام الفحوصات البكتريولوجية التقليدية. وقد تم اختبار حساسية الجراثيم المعزولة لعدد من أنواع المضادات

الحيوية. أظهرت النتائج إن العدد الكلي للجراثيم المعزولة هو 69 جرثومة. و قد كانت الجراثيم السالبة لصبغة كرام (82.6%) الأكثر تكرارا من الجراثيم الموجبة لصبغة كرام (17.4%) من بين جميع العينات. كانت جرثومة *E.coli* هي الأكثر شيوعا من بين الجراثيم السالبة لصبغة كرام (2. 23%)، تلتها *Pseudomonas* spp. (7. 21%)، *K. pneumoniae* (14.5%)، *Proteus* spp.(11.6%)، *Acinetobacter* spp. (8.7%) و *E.aerogenes* (2.9%). وكانت جرثومة *S.aureus* (7.2%) هي الأكثر شيوعا من بين الجراثيم الموجبة لصبغة كرام، تبعتها *E.faecalis* (5.8%) و *S.epidermidis* (4.3%). أظهرت الدراسة أن المضادات الحيوية الأكثر فعالية هي اميكاسين وسيبروفلوكساسين و فانكومايسين وجينتامايسين وكلورامفينيكول وازيثرومايسين.

الكلمات الدالة: وحدات العناية المركزة، العزلات الجرثومية، نمط التحسس للمضادات الحيوية.

INTRODUCTION

Nosocomial infection (NI) is defined as an infection acquired by a patient in a hospital or other healthcare facility that was not present or incubating at the time of admission, or that was the residual of an infection acquired during a previous admission (WHO, 2002). The time frame for diagnosis of a NI will clearly be dependent on the incubation period of the specific infection, thus, 48 hours after admission is generally deemed indicative of nosocomial rather than community acquired infection (Garner *et al*.,1988).

Although 5% to 10% of all patients are treated in intensive care units (ICUs), they constitute about 25% of all NIs and the incidence is 5 to 10 times higher than in general hospital wards (Al - Johani *et al.*, 2010), which means that NIs are specially prominent in ICU (Ewans *et al.*, 1999). This may be related to the use of large numbers of invasive monitoring devices, endotracheal and tracheostomy tubes, in addition to patients factors including extremes of age, immunocompromised status, malnutrition, severe underlying disease, wide use of antibiotics and to a high incidence of cross infection (Weber *et al.*, 1999).

The three most common NIs are ventilator-associated pneumonias, urinary tract infections, and blood stream infections (Richards *et al.*, 1999).

There is a wide diversity between institutions in the prevalence of pathogens and in their antimicrobial susceptibility (Fridkin, 2001). Therefore, this study aims to determine the types and frequencies of bacterial isolates from patients with ICUs-acquired infections, as well as the susceptibility patterns of these bacterial isolates to selected antibiotics which is essential to produce empirical antibiotic protocols for individual ICU.

PATIENTS AND METHODS

This study was approved by the scientific research committee at the College of Medicine, University of Mosul. Formal consent was taken from all patients after careful examination.

The present work was carried out in the Diagnostic Bacteriology Laboratory, Department of Microbiology, College of Medicine, University of Mosul during the period from December 2010 to June 2011.

The subjects enrolled in the current study were composed of one hundred and one patients admitted into three ICUs, including Cardiac Care Unit (CCU), Critical and Respiratory Care unit (RCU) in Ibn Sinna Teaching Hospital and Surgical Care Unit (SCU) in Aljamhori Teaching Hospital. These patients developed clinical suggestion of new infections of urinary tract, lower respiratory tract or wound following the 48 hours of their admission to the ICUs.

Samples, if present, were collected from patients at the time of their admission to ICUs or during the first 48 hours of their admission if there was clinical suggestion of an infection. Any positive finding during this period was considered as a pre-existing infection and the results were not included in this study. This was done just to differentiate the new infection from the pre-existing one. In addition, any patient died or was discharged before passing 48 hours on his admission to the ICU was excluded from the study. In case of readmisson of patient to the ICU, he was considered as a new case.

A total of 154 clinical samples were collected from the studied patients following the 48 hours of their admission in the above mentioned three ICUs. The specimens consisted of 83(53.9%) urine samples, 44 (28.6%) respiratory samples, and 27(17.5%) wound samples. After collection of the clinical samples, they were transferred to the laboratory without undue delay for processing, and inoculation. Different culture media were used for inoculation including sheep blood agar (5%), Chocolate agar, MacConkey's agar, and Nutrient agar (Oxoid, UK). All media were incubated at 37°C for 24 hours, with further 24 hours incubation if there was no growth.

The microorganisms were identified by the conventional bacteriological methods depending on Gram's stain, cultural characteristics, and standard biochemical tests. Furthermore, the susceptibility of the bacterial isolates were determined against selected antibacterial agents using discs diffusion method (Bauer *et al.*, 1966) on Mueller-Hinton agar (Oxoid, UK).

STATISTICAL ANALYSIS

Statistical analysis was performed by using Mini Tab version 13. Chi-square was used to evaluate the present data. Statistically test results were considered significant at p-value ≤ 0.05 (Kirkwood, 1988).

RESULTS

The studied patients composed of 52(51.5 %) females and 49(48.5%) males.

The age of the patients ranged from 5-85 years (mean 52.5 ± 16.8 SD), whereas, the duration of stay of these patients in the ICUs ranged from 3-32 days (mean 7.1 ± 4.6 SD).

The samples were collected from 35(34.7%) patients of CCU, 33(32.7%) patients of RCU, and 33(32.7%) patients of SCU.

Out of the total 101 studied patients, 53(52.5%) had ICUs acquired bacterial infections. From these 53 infected patients, 39(73.6%) individuals developed only one infection, while the rest 14(26.4%) developed more than one type of infection. The remaining 48(47.5%) patients showed negative bacterial growth. Yeasts were isolated from 21(20.8%) of them. The rest 27(26.7%) patients did not show any defined growth (Fig. 1).

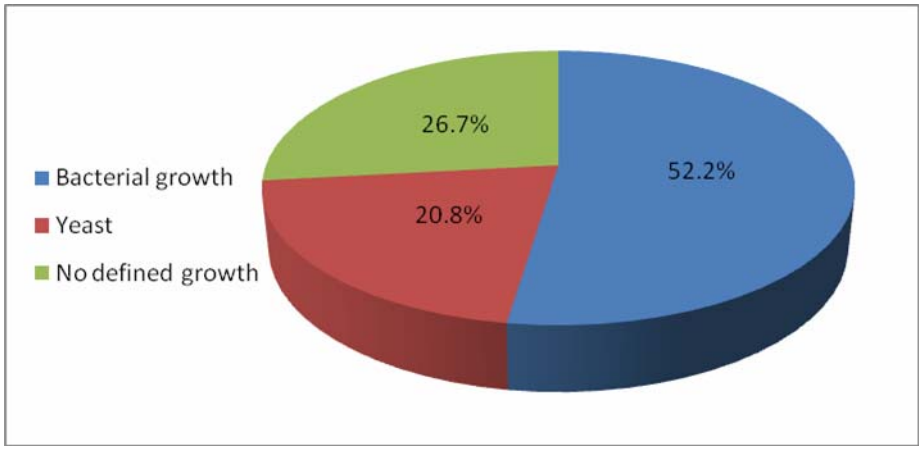

Fig. 1: The comprehensive results of culture technique

Out of the 67 samples which yielded positive cultures, 65(97%) showed monomicrobial infections, while polymicrobial infections were detected in 2(3%) samples only.

The total number of the isolated bacteria was 69 of which Gram-negative bacteria were more frequently encountered (57, 82.6%) than the Gram-positive ones (12, 17.4%). The predominant isolate for Gram-negative bacteria was *E.coli* 16(23.2%), followed by *Pseudomonas* spp. 15(21.7%), where *P.aeruginosa* comprised 10 (66.7%), *K.pneumoniae* 10(14.5%), *Proteus* spp. 8(11.6%), where *P.mirabilis* constituted 6(75%) and *P.vulgaris* 2(25%), *Acinetobacter* spp. 6(8.7%), *E.aerogenes* 2(2.9%). On the other hand, the predominant Gram-positive bacterial isolate was *S.aureus* 5(7.2%), followed by *E.faecalis* 4(5.8%), and *S.epidermidis* 3(4.3%) as shown in Table 1.

Table 1: The total number of bacteria isolated from the three types of infection.

Bacterial isolates	**Type of infection**			**Total**
	RTIs No. (%)	**UTIs No. (%)**	**Wound infection No. (%)**	**No. (%)**
Gram-negative				
E.coli	_	9 (28.1)	7 (46.7)	16 (23.2)
Pseudomonas Spp.	8 (36.4)	4 (12.5)	3 (20)	15 (21.7)
K.pneumoniae	6 (27.3)	4 (12.5)	_	10 (14.5)
Proteus spp.	1 (4.5)	5 (15.6)	2 (13.3)	8 (11.6)
Acinetobacter spp.	4 (18.2)	2 (6.3)	_	6 (8.7)
E.aerogenes	_	2 (6.3)	_	2 (2.9)
Subtotal	19 (86.4)	26 (81.3)	12 (80)	57 (82.6)
Gram-positive				
S.aureus	2 (9.1)	1 (3.1)	2 (13.3)	5 (7.2)
E.faecalis	1 (4.5)	2 (6.3)	1 (6.7)	4 (5.8)
S.epidermidis	_	3 (9.4)	_	3 (4.3)
Subtotal	3 (13.6)	6 (18.8)	3 (20)	12 (17.4)
Total	22 (31.9)	32 (46.4)	15 (21.7)	69 (100)

Statistically there was significant difference between the isolated number of Gram-negative and Gram-positive bacteria where the P-value was < 0.05.

The antibiogram profiles of the isolates are shown in figure 2 and 3. Amikacin (74.3%), followed by ciprofloxacin (71.5%), chloramphenicol (66%), and tobramycin (65.6%) are the effective agents against Gram-negative bacteria. Conversely, the least effective antibiotic was co-amoxiclav (0%). In addition, the sensitivity of *Pseudomonas* spp. to piperacillin and ticarcillin were 40% and 13.3% respectively. Moreover, the sensitivity of *Acinetobacter* spp. to colistin was 100% .

On the other hand, the most effective antibiotics against Gram-positive bacteria were, vancomycin (100%), amikacin (100%), gentamicin (100%), chloramphenicol (100%), followed by azithromycin (73.9%).

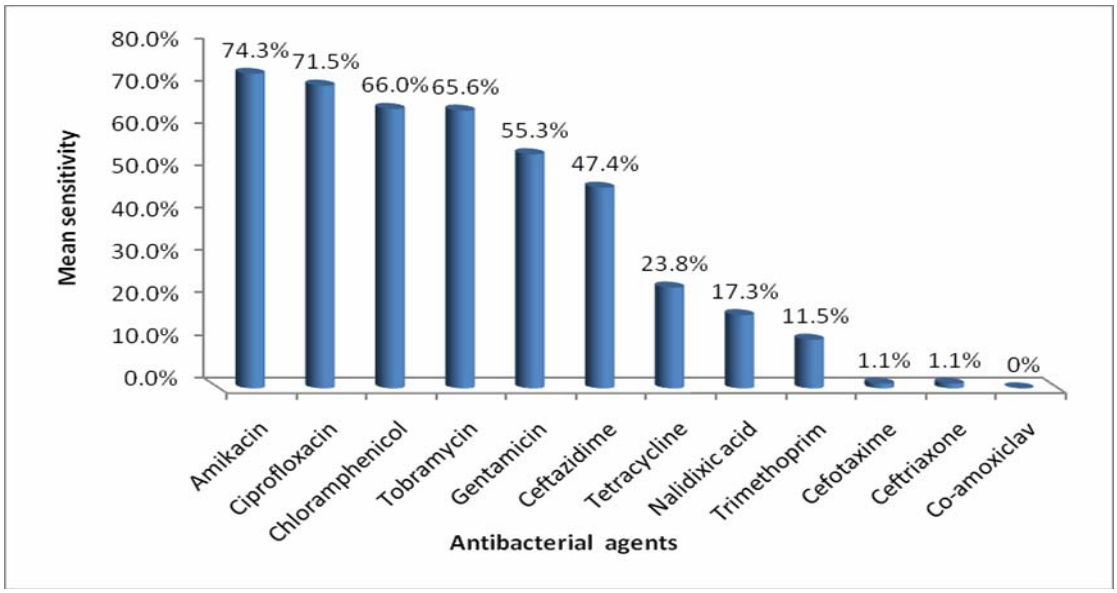

Fig. 2: Mean sensitivity percentages of Gram-negative isolates to twelve antibacterial agents.

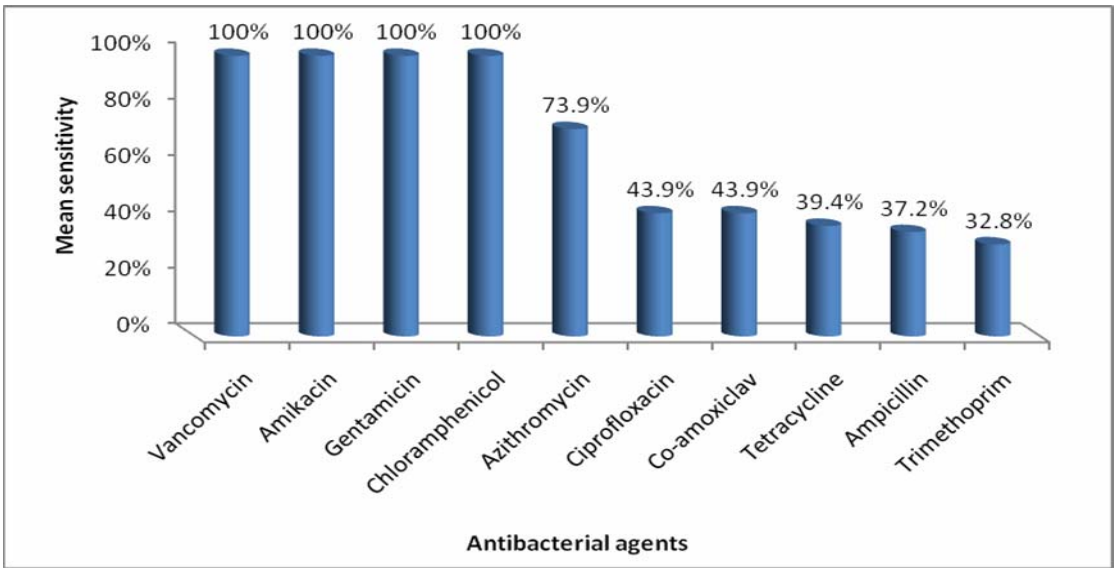

Fig. 3: Mean sensitivity percentages of Gram-positive isolates to ten antibacterial agents.

DISCUSSION

NIs in ICUs have become increasingly problematic in the recent years, nevertheless, no records about these infections are available in our area, hence, this study was carried out to assess this problem. In the current study, 52.5% of the examined patients were supported by positive bacterial cultures. This result was in agreement with that of another study(Hassanzadeh *et al.*, 2009) in which 51.7% of the total examined patient showed NI based on culture and clinical findings.

The possible explanations for the negative bacterial culture yielded by the remaining 47.5% of individuals are either those patients had no NIs, or the causative agents could be of atypical microorganisms that cannot grow on the traditionally used bacteriological culture media. Furthermore, urine and sputum cultures of 20.8% of the examined patients yielded yeast cells (*Candida* spp.), and this result is similar to that (22%) mentioned by Tennant *et al*., (2005). This finding may be due in part to the immunocompromised state of such patients and the use of broad-spectrum antibiotics.

Out of the total 52.5% nosocomially infected individuals, 73.6% had only one sort of infection, while the rest 26.4% developed more than one type of infection. These findings were in contrast to that of another study(Hassanzadeh *et al.*, 2009) in which 80.4% of the infected patients had more than one type of infection while the remaining 19.6% developed one infection only.The development of more than one infection in a single individual may reflects the presence of a severe underlying disease, or may be due to other factors that cause immunocompromisation.

In this study, out of the total 69 bacterial isolates, Gram-negative bacteria were more significantly involved in infections (57, 82.6%) than were Gram-positive bacteria (12, 17.4%). This predominance of Gram-negative bacteria was coincident with the finding of some recent studies(Hassanzadeh *et al.*, 2009; Nicoletti *et al.*, 2006), which may be due to their wide prevalence in the hospital environment, and most of them represented a wide range of normal flora. In addition, their frequent resistance to antibiotic may play a role in their persistence and spread.

The *Enterobacteriaceae* represented the most frequently isolated pathogens in the current work (52%), *E.coli* (23.2%), *K.pneumoniae* (14.5%), *Proteus* spp. (11.6%), and *E.aerogenes* (2.9%). In addition, *Pseudomonas* spp. (21.7%) ranked second to *E.coli*. This finding was in contrast to that of Ponce de Leon-Rosales *et al*., (2000) in which *Enterobacteriaceae* represented 25.9%, and *Pseudomonas* spp. constituted 17.2%. However, the members of *Enterobacteriaceae* are widely diffused in the human body as normal flora, which make them one of the most important available endogenous bacteria ready for NI in immunocompromised patients. While *Pseudomonas* spp. are widely distributed in the environment, in the water, and even in some medical instruments and machines as the ventilator circuit.

Other Gram-negative isolates were *Acinetobacter* spp., in this study they represented 8.7% of the bacterial isolate, while in other studies they constituted 11.9% (Erbay *et al.*, 2003), 14.6% (Salomao *et al.*, 2008), and 26.8% (Meric *et al.*, 2005). Thus, there are a clear regional differences and variation in the prevalence of *Acinetobacter* spp.. This may be due to the variation in the local infection control measures which may alter the infection rates.

Concerning the Gram-positive bacteria, the predominant isolate was *S.aureus* which represented 7.2% of the total bacterial isolates, followed by *E.faecalis* (5.8%), and

S.epidermidis (4.3%). In another study (Erbay *et al.*, 2003) the predominant Gram-positive microorganism was *S. aureus* (11.3%), and CONS (8.4%). While another investigator reported a percentages of 20% and 18.7% for CONS and *E. faecalis* respectively which were the most frequent isolates of Gram-positive microorganisms (Tennant *et al*., 2005).

The overall decrease in the percentage of Gram-positive bacteria in comparison to the Gram-negative bacteria may be due to the direction of empirical therapy towards the Gram-positive one, in the time that the Gram-negative bacteria show resistance to this therapy.

However, the distribution of pathogenic microorganisms tends to vary among the different ICU studies (Fridkin, 2001 ; Meric *et al.*, 2005).

The isolated Gram-negative bacteria showed high resistance to many of the tested agents, including trimethoprim, ceftriaxone, cefotaxime, and co-amoxiclav. This finding was in accordance with that of other studies(Goel *et al.*, 2009 ; Taher and Golestanpour, 2009) in which there was an alarmingly high rate of resistance to cephalosporin and Beta-lactam-Beta-lactamase inhibitor group of drugs. This high rate of resistance might be due to the selective influence of extensive usage of these drugs.

On the other hand, the most effective antibiotic against Gram-negative bacteria including *Pseudomona*s spp. was amikacin, followed by ciprofloxacin, chloramphenicol, tobramycin, gentamicin, and ceftazidime. This result was in agreement with that of other studies(Hassanzadeh *et al.*, 2009; Taher and Golestanpour, 2009)in which amikacin, ciprofloxacin, and ceftazidime were the most active agents against the Gram-negative isolates.

The sensitivity of *Acinetobacter* to colistin was 100%. This result was consistent with that of other studies (Urban *et al.*, 2003; Linden and Paterson, 2006) in which colistin was the most active agent.

Regarding the Gram-positive bacteria, all isolates of *S.aureus*, *S.epidermidis*, and *E.faecalis* showed a full sensitivity to vancomycin, amikacin, gentamicin, and chloramphenicol. This high level of sensitivity may be due to the less frequent use of such antibiotics in hospitals. Also, azithromycin had a good activity (73.9%) against these types of bacteria.

CONCLUSIONS

The Gram-negative bacteria were the major cause of infections in the ICUs. The commonest isolates were *E. coli*, *Pseudomonas* spp., and *K. pneumoniae*. The best empirical therapy should include amikacin or ciprofloxacin with vancomycin or azithromycin which will provide an adequate coverage while waiting for culture and sensitivity results.

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