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Determination of Antimicrobial Drug Resistance among Bacterial Isolates in Two Hospitals of Baghdad

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ABSTRACT

Objective

To evaluate the resistance of clinical isolates from two main hospitals in Baghdad, Iraq, against commonly used antimicrobial drugs.

Methods

Five hundred clinical samples were collected from various sources at two hospitals in Baghdad and subjected to establish microbiological methods to determine their sensitivity to commonly used antimicrobial drugs. The antimicrobial sensitivity test used was the Kirby-Bauer disc diffusion method. Interpretations of the test outcomes were according to international values.

Results

Out of 500 clinical specimens, it was possible to obtain 239 bacterial isolates. The predominant isolates (74 specimens; 31%) were from throat swabs from which 40 isolates were of GAβHS followed by 16 of *Klebsiella pneumonia*e. The second group of isolates were from blood (67 specimens; 28%) in which *Staphylococcus aureus* was represented by 20 specimens followed by *Proteus* species by 16 specimens. The third group of isolates was from the urine specimens (42 specimens; 17.6%). The urine isolates were distributed as *Proteus* spp (20 specimens) followed by bacterial isolates of *Pseudomonas aeruginosa* and *K. pneumoniae* (8 specimens each). The fourth group of isolates was from sputum (40 specimens; 16.7%) in which GAβHS represented 18 isolates followed by 12 isolates of *K. pneumonia*e. No *Proteus* spp was isolated from either sputum or purulent wounds. Similarly, no GAβHS and *K. pneumoniae* were isolated from purulent wounds. The results of the antimicrobial resistance tests among the bacterial isolates revealed that all isolates were highly resistant to most of the drugs used in this study. GAβHS was resistant to all of the drugs except for Cefotaxime (76.7%). *Ps. aeruginosa* isolates were completely resistant to Cefotaxime, Cephalexin and Amoxicillin.

Conclusion

From this study it is concluded that multiple-resistant bacteria isolates are common and that antimicrobial resistance is widespread in Iraq. A policy to overcome this crisis will be urgently needed.

Keywords: Multi-Drug Resistance, Antimicrobial Agents, Antibiotic Sensitivity Test.

INTRODUCTION

Bacterial resistance to antimicrobial drugs is increasing due to either abuse of the drugs or genetic changes in the micro-organisms (1-3). This resistance creates a major problem to the public health sector and leads to difficulties in the treatment of a wide range of bacterial diseases. This resistance is far from being controlled (4-8). Infections resulting from resistant bacteria have been shown to be more frequently associated with increased morbidity and

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mortality as compared to those caused by susceptible pathogens (9, 10). The World Health Organization warned the international community that drug-resistant bacteria are emerging worldwide and becoming a real challenge to health care and if no immediate action is taken antimicrobial drugs may lose their power to cure diseases (11, 12). Drug-resistant bacteria cause serious nosocomial and community acquired infections that are hard to eradicate by using available drug (11, 12). Antibiotic resistance leads to prolonged hospital stay and increases the cost in terms of treatment and is life threatening (13-15). Antimicrobial agents are the most commonly used and misused among all drugs and the consequences of the wide spread use of antimicrobial drugs has increased the emergence of antibiotic resistant pathogens which necessitate the need for new drugs (16-21). Reducing inappropriate antibiotic use is thought to be the best way to control the emergence of resistance. The aim of the present study is to evaluate the resistance level of clinical bacterial isolates to commonly used antibiotics in Baghdad hospitals.

MATERIALS AND METHODS

This study was conducted over a period of one year, from October 2014 to October 2015. Five hundred clinical specimens were collected from two hospitals (Al-Yarmouk Teaching Hospital and Ibn Al-Nafees Hospital) in Baghdad.

Source of specimens: Urine, blood, throat swabs, sputum and purulent wounds. Samples were collected from patients referred to the laboratory from different departments of the two above mentioned hospitals.

Processing of samples: All samples were subjected to established microbiological methods for final and accurate diagnosis.

Antimicrobial disc susceptibility test: Testing all of the isolates to the commonly used antimicrobials agents was performed according to the Kirby-Bauer disc diffusion method on Muller-Hinton agar media (22). The inhibition zone sizes were interpreted according to Kirby-Bauer method values (Table 1).

STATISTICAL ANALYSIS

All data entered and analyzed using the SPSS® software (version 16.0; SPSS, Inc, Chicago, IL). Analysis of variance (ANOVA) was used to test the hypothesis that there is difference in the resistance among different strains. For statistical analysis p-value was two-sided and p-value of 0.05 or less was considered statistically significant.

Table1. Interpretation of zone inhibition using Kirby-Bauer method (disc diffusion method).

Antimicrobial agent	Code	Disc potency µg/Disc	Diameter of zone inhibition (mm)		
			Resistant	intermediate	Sensitive
Ampicillin	AM	10	≤11	12-13	≥20
Cefotaxime	CTX	30	≤14	15-22	≥23
Cephalexin	KF	30	≤14	15-17	≥18
Chloramphenicol	C	30	≤12	13-17	≥18
Ciprofloxacin	CIP	10	≤15	16=20	≥21
Clindamycin	CN	2	≤12	13=17	≥18
Tobramycin	TM	10	≤13	13-14	≥15
Erythromycin	E	15	≤13	14-17	≥18
Ampilox*	AMP	30	≤14	15-16	≥17
Gentamycin	GN	10	≤12	13-14	≥15
Nalidixic acid	NAL	30	≤13	14-18	≥19
Penicillin-G	PG	6	≤20	21-28	≥29

Antimicrobial agent	Code	Disc potency µg/Disc	Diameter of zone inhibition (mm)		
			Resistant	intermediate	Sensitive
Rifampicin	**RA**	**5**	**≤16**	**17-19**	**≥20**
Co-Trimoxazole	**SXT**	**25**	**≤18**	**19-2**	**24-32**
Amoxicillin	**AMX**	**10**	**≤19**	**-**	**≥29**
Amikacin	**AN**	**30**	**≤14**	**15-16**	**≥17**

***Ampilox : Ampicillin and Cloxacillin**

RESULTS

In the present study a total of 239 (47.8%) bacterial isolates were obtained out of 500 clinical specimens of urine, blood, throat swab, sputum and purulent wounds collected from two hospitals at Baghdad city. The distribution of each type of bacterial isolates according to their sources is shown in Table 2.

Table 2 shows that GAβHS is the dominant pathogen with 70 isolates specimens representing 29.3% of the total specimens. *K. pneumoniae, S. aureus* and *Proteus* spp isolates are represented in 19.7% (47 specimens), 19.2% (46 specimens) and 17.6% (42 specimens) of the total samples, respectively. *Ps. aeruginosa* is the least found pathogen represented in 14.2% (34 specimens) of the samples.

Urine samples show a predominance of *Proteus* spp isolates with 47.6% (20 specimens) of the samples. *K. pneumoniae, Ps. aeruginosa* and *S. aureus* are present in a lesser number of specimens representing 19% (8 specimens), 19% (8 specimens) and 14.3% (6 specimens) respectively. GAβHS is notably absent in the urine samples.

In the blood samples all studied pathogens are present with percentages ranging from 11.9 (*Ps. aeruginosa*) to 29.9 (*S. aureus*) of the samples.

Throat swabs show a predominance of GAβHS with 54% of the isolates (40 samples). *K. pneumoniae* isolates represent 21.7% (16 specimens), whereas *S. aureus, Proteus* spp and *Ps. aeruginosa* represent 10.8%, 8.1% and 5.4% of the throat swab samples.

The sputum samples are similarly predominated by GAβHS with 45% of the isolates (18 samples) followed by *K. pneumoniae* (30%), *Ps. aeruginosa* (15%) and *S. aureus* (10%). *Proteus* spp was not found in the sputum samples.

Purulent wounds showed only *S. aureus* and *Ps. aeruginosa* pathogens with 50% (8 specimens) of the samples each. *Proteus* spp, GAβHS and *K. pneumoniae* are absent in the purulent wounds samples.

The distribution of the pathogen isolates in the different types of samples is also shown in Table 2. *Proteus* spp is the most prevalent species in urine samples, second most abundant in blood samples, of minor presence in throat swabs and is absent in the sputum and purulent wounds samples. GAβHS is most prevalent group in the throat swab and sputum samples, of moderate presence in the blood samples and is absent in the urine and purulent wounds samples. *S. aureus* is the most common pathogen in the blood and purulent wounds samples and is of lesser presence in the urine, sputum and throat swabs samples. *Ps. aeruginosa* is most prevalent in the purulent wounds samples, of moderate presence in the urine samples and is of lesser presence in the throat swabs, blood and sputum samples. *K. pneumoniae* is the second most common pathogen in the sputum and throat swabs samples, moderately represented in the urine and blood samples and is absent in the purulent samples.

Table 2. Species and percentage of bacterial isolates according to source of samples

Source of specimens	Bacterial isolates						
	***Proteus*spp**	**GAβHS**	***S. aureus***	***Ps. aeruginosa***	***K. pneumoniae***	**Total**	**%**
Urine	20 (47.6%)	- (0.0%)	6 (14.3%)	8 (19%)	8 (19%)	42	17.6
Blood	16 (23.9%)	12 (17.9%)	20 (29.9%)	8 (11.9%)	11 (16.4%)	67	28
Throat swabs	6 (8.1%)	40 (54%)	8 (10.8%)	4 (5.4%)	16 (21.7%)	74	31
Sputum	- (0.0%)	18 (45%)	4 (10%)	6 (15%)	12 (30%)	40	16.7
Purulent wounds	- (0.0%)	- (0.0%)	8 (50%)	8 (50%)	- (0.0%)	16	6.7
Total	42 (17.6%)	70 (29.3%)	46 (19.2%)	34 (14.2%)	47 (19.7%)	239	100

The pattern of resistance of bacterial isolates to commonly used antimicrobial drugs is given in Table 3. All isolates are highly resistant to most of the antimicrobials drugs. GAβHS were completely resistant (100%) to all the drugs used in this study except Cefotaxime (72.2%). This result demonstrates a multi-drug resistance. The bacterial isolates of *Ps. aeroginosa* showed complete drug resistance (100%) to Cefotaxime, Cephalexin and Amoxicillin and the resistance to the remaining drugs ranged between 61.7% and 85.7%. The other pathogen which showed a high resistance to most of the drugs was *K. pneumoniae.* This pathogen isolate showed a high resistance to Ampilox (93.6%), Cefotaxime (89.4%), Amoxicillin (89.4%), Tobramycin (82.9%) and Cephalexin (82.9%).

High to moderate resistance was shown by *S. aureus* isolates (98% - 61.7%) to all of the used antimicrobial drugs except for the drug Ciprofloxacin to which the isolate showed a very low resistance (17.4%).

Proteus spp isolates also showed high to moderate resistance to most of the tested antibiotics except for two drugs to which the pathogen showed relatively low resistance; namely Amikacin and Penicillin G (46.8%).

ANOVA was used in the present study to test if there was a significant difference in the resistance among different strains. Results show that there was a significant difference in strains resistance against different antibiotics (*p*-value 0.014)

Table 3. Percentages (%) of bacterial isolates which showed resistance to the antimicrobial agents.

Antimicrobial Agent	Types of the isolated bacteria				
	Proteus spp	**GAβHS**	***S. aureus***	***Ps. aeruginosa***	***K. pneumoniae***
Cefotaxime (CTX)	**89.4**	**76.2**	**95.6**	**100**	**89.4**
Cephalexin (KF)	**74.5**	**100**	**95.6**	**100**	**82.9**

Antimicrobial Agent	Types of the isolated bacteria				
	Proteus spp	GAβHS	*S. aureus*	*Ps. aeruginosa*	*K. pneumoniae*
Amikacin (AM)	**46.8**	**100**	**98**	**78.6**	**74.5**
Cotrimoxazole (SXT)	**70.2**	**100**	**87**	**85.7**	**76.6**
Penicillin G (PG)	**46.8**	**100**	**97.8**	**70.2**	**85**
Ampilox (AMP)	**89.4**	**100**	**78**	**64.3**	**93.6**
Gentamycin (GM)	**53.2**	**100**	**73.9**	**76.6**	**59.6**
Amoxicillin (AMX)	**66**	**100**	**86.5**	**100**	**89.4**
Tobramycin (TM)	**88**	**100**	**93**	**68**	**82.9**
Ciprofloxacin (CIP)	**83.4**	**100**	**17.4**	**61.7**	**46.8**

DISCUSSION

The present study provides information on the distribution of pathogenic bacteria isolates from clinical specimens collected from two main hospitals in Baghdad city and their resistance to commonly used antimicrobial drugs.

The results reveal that GAβHS is the most prevalent bacterial isolate and is involved in many bacterial infections especially those of the upper respiratory tract. The present findings are in agreement with those reported by many authors concerning the responsibility of these isolates with the above mention infections (23-26). Nevertheless, no GAβHS isolates were recovered from either urine or pus.

*K. pneumonia*e which has recently become an important pathogen in nosocomial infections and is recovered from respiratory tract, urinary tract infections (UTI) and pus cases (13, 14, 21). The findings of the present study show that *K. pneumonia*e is the second leading bacterial isolate and is recovered from throat swabs, sputum, urine and blood, but was not found in purulent wounds specimens. With the exception of the absence of *K. pneumonia*e in the purulent wounds, these results are similar to those reported by other workers (22, 25).

Proteus spp were most common in isolates from urine and blood. These results are in accordance with the findings that this pathogen is associated with UTI and bacteremia (15).

S. aureus isolates which are encountered as hospital acquired infection and are responsible for infections such as sepsis, pneumonia and wounds infections (12, 13, 20), represent the highest occurrence in purulent wounds (50%). Our results are mostly in agreement with those obtained by other authors (24-28).

Interestingly, *Ps. aeruginosa* isolates were least prevalent in this study although this microorganism emerged recently as an important pathogen responsible for nosocomial infections (12). However, it was found in all types of specimens examined.

In the present study the results of the antimicrobial susceptibility obtained using the Kirby- Bauer disk diffusion method for the bacterial isolates revealed that the resistance to the commonly used antimicrobial drugs as

manifested in the tested bacterial isolates can be considered very high. All isolates were resistant to most of the drugs used in this study. Development of antibiotic resistance in bacteria represents a problem of great concern for those involved in field of medicine.

The pattern of susceptibility in our work revealed that GAβHS is completely (100%) resistant to all drugs used in this study with the exception of Cefotaxime to which this pathogen is slightly less resistant (76.2%). This pathogen group appears to have become multi-drug resistant. Similar findings have been reported by other authors (29-34). Speculation can be given here if β-lactamase or plasmids or other genetic mechanism factors are playing roles in this situation, but it needs further investigations. The importance of this result is that GAβHS has been associated with a wide range of diseases (34). These results indicate that no drug from those tested in this study can be used for treatment of infection caused by GAβHS.

Similarly, *S. aureus* which is responsible for a wide range of infection especially those which are hospital acquired (12, 13, 27) exhibited high resistance to almost all of the tested antibiotics. The pattern of resistance of *S. aureus* (Table 3) revealed that it is highly resistant to Amikacin (98%), Penicillin G (97.8%), both Cefotaxime and Cephalexin (95.6%), Tobramycin (93), Cotrimoxazole (87), Amoxicillin (86.5), Ampilox (78) and Gentamycin (73.9). However, resistance against Ciprofloxacin is low (17.4%). It is not known whether this resistance is due to the production of β-lactamases or due to R-plasmids. Our results of *Staphylococcus* resistance seems to be higher than those demonstrated by other researchers (21, 27, 31). This could be due to geographical distribution or to drug abuse in the study area. Accordingly it is very important to find the correct policy for finding the drug of choice for treating infection caused by *Staphylococcus* infection. From our results it seems that the only drug which can be used against *S. aureus* is Ciprofloxacin.

Ps. aeruginosa is becoming resistant to commonly used antibiotics and antiseptics and has the ability to establish itself widely in hospitals and gaining more and more resistance to newer antibiotics (21, 25, 31, 35). Our results (Table 3) reveled that *Ps. aeruginosa* is completely resistant (100%) to Cefotaxime, Cephalexin and Amoxicillin and is highly resistant to the remaining tested drugs where the least level of resistance is to Ciprofloxacin (61.7%).

*K. pneumonia*e has been associated with many different types of infections and one of the important aspects of *Klebsiella* associated infections is the emergence of multi-drug resistant strains particularly those involved in nosocomial diseases (9, 10, 21, 29). Table 3 shows that *K. pneumonia*e is highly to moderately resistant to the tested drugs except for Ciprofloxacin to which low resistance (46.8%) is indicated. Our results are not in agreement with other results (12) which found that *K. pneumonia*e isolates from a hospital in Gujarat (India) are highly susceptible to the drugs Gentamycin and Amikacin whilst our results showed that *K. pneumoniae* isolates are resistant to those drugs at the level of (59.6%) and (74.5%) respectively.

Proteus spp are involved in UTI and formerly it has been suggested that Cefotaxime is the drug of choice while in our results and as can be seen from Table 3 *Proteus* spp are highly resistant (85.4%) to this drug.

Multi-drug resistant bacteria cause serious nosocomial and community acquired infections that is hard to control by classical available methods. The present study shows that all clinical isolates are becoming resistant to the commonly used antimicrobials. This is mostly due to abuse of the drugs and that practitioners prescribe treatment on the basis of their experiences without laboratory tests. To control these problems antibiotic policies should be formulated to resist, overcome and prevent the spread of resistant organisms.

CONCLUSION

Bacterial isolates from urine, blood, throat swab, sputum and purulent wounds samples collected from Baghdad hospitals included the following species of bacteria: GAβHS, *K. pneumoniae, S. aureus, Proteus* spp and *Ps. aeruginosa.* Testing the isolates to the commonly

used antimicrobials agents revealed that the above bacteria have developed mostly a high degree of multi-drug resistance. Therefore there is an urgent need to formulate a policy to overcome this crisis and prevent the spread of multi-drug resistance to antimicrobials.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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تحديد مقاومة الأدوية المضادة للميكروبات بين العزلات البكتيرية في اثنتين من مستشفيات بغداد

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ملخص

الهدف

تقييم مقاومة العزلات السريرية من مستشفيين رئيسيين في بغداد، العراق، للمضادات الحيوية شائعة الاستخدام.

المنهج البحثي

تم جمع خمسمائة عينة سريرية من مصادر مختلفة في مستشفيين في بغداد. فحصت العينات لتحديد مدى حساسية الأدوية المضادة للميكروبات المستخدمة بشكل شائع. بطريقة Kirby Bauer. تم تفسير نتائج الاختبار وفقا للقيم الدولية.

النتائج

من بين 500 عينة سريرية، تم الحصول على 239 عزلة بكتيرية. وكانت العزلات السائدة من مسحات الحلق 74 (31٪) منها 40 (54٪) عزلات GAβHS تليها 16 (16.5٪) K. pneumoniae. وكانت العزلات الثانية من الدم 67 (28٪) من العزلات البكتيرية شكلت S. aureus 20 (29.9٪) تليها 16 (23.9٪) Proteus spp. .

أما عزلات البول 42 عزلة (17.6٪) توزعت كالتالي 20 (47.6٪) Proteus spp تليها 8 (19٪) من العزلات البكتيرية من Ps. aeruginosa و K. pneumoniae على التوالي. ومن بين 40 عزلة (16.7٪) من البلغم وجد ان GAβHS تمثل 18 (45٪) عزلات تليها 12 (30٪) عزلات من K. pneumoniae . لم يتم عزل Proteus spp من البلغم أو الجروح الصديدية. وبالمثل، لم يتم عزل أي من GAβHS و K. pneumoniae من الجروح القيحية.

أظهرت نتائج اختبارات مقاومة مضادات الميكروبات بين العزلات البكتيرية أن جميع العزلات كانت شديدة المقاومة لمعظم الأدوية المستخدمة في هذه الدراسة. كانت GAβHS مقاومة لجميع الأدوية باستثناء Cefotaxime (76.7٪). Ps. aeruginosa كانت العزلات مقاومة تماما ل Cefotaxime، Cephalexin و Amoxicillin

الاستنتاج

استنتج من هذه الدراسة أن عزلات البكتيريا ذات المقاومة المتعددة للمضادات الحيوية (MDR) شائعة، وأن مقاومة مضادات الميكروبات منتشرة على نطاق واسع مما يتطلب وضع سياسة للتغلب على سوء استعمال المضادات الحيوية في العراق.

الكلمات الدالة: مقاومة الأدوية المتعددة، العوامل المضادة للميكروبات، اختبار حساسية المضادات الحيوية.

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