RESEARCH ARTICLE

Resistance Rates of the most Isolated Bacteria from different Clinical Samples, Kerbala, Iraq

Maysaa Saleh Mahdi[1], Suhad Hadi Mohammed[1*], Mohanad Mohsin Ahmed[2], Ali Najm Al-Deen[1], Nargis Fadhil[1], Noor Yahya Abid Zaid[1], Ibrahim Hayder Jasim Alkhafaji[3]

[1]*Faculty of Applied Medical Sciences, Department of Clinical Laboratories, Kerbala University, University Street 11252, Kerbala, Iraq*

[2]*Faculty of Medicine, Department of Microbiology, Kerbala University, University Street 11252, Kerbala, Iraq*

[3]*Faculty of Pharmacy, Kerbala University, University Street 11252, Kerbala, Iraq*

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ABSTRACT

Determining the bacterial causative agents of infections by identifying their antimicrobial patterns will enable health institutions to limit the unnecessary use of antibiotics, and take active ways in preventing the spread of drug-resistant bacteria. This study aimed to identify the most common bacterial isolates responsible for infection and their antibiotic resistance rates. The results showed that *Escherichia coli*, *Staphylococcus aureus* (*S aureus*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) represent the most common bacteria isolated with a percentage of 23.9, 18.8, and 16.2%, respectively. High resistance rates were found for the most common bacterial isolates. Other important findings are the presence of extended-spectrum B-lactamase (ESBL) producing bacteria and the appearance of hetero-resistance phenomenon. Moreover, the bacterial infection is mainly occurring in men. No significant correlation was observed in the type of isolated bacteria with patient admission status.

E. coli strains were found to be highly resistant to amoxicillin-clavulanic acid, ceftriaxone (88.9%), ceftazidime (85.2%), trimethoprim-sulfamethoxazole (74.1%), and ciprofloxacin (59.3%). Whereas, the highest sensitivity rates were seen with meropenem antibiotic (92.6%). Concerning *S. aureus* isolates, 100, and approximately 91% of resistant rates were seen to penicillin and cefoxitin, respectively [methicillin-resistant *S. aureus* (MRSA)]. Approximately 50% of MRSA were vancomycin-resistant *S. aureus* (VRSA). Resistant rates of *P. aeruginosa* isolates to gentamycin and ciprofloxacin were 47.1%, amikacin 41.2%, and levofloxacin 35.3%. In conclusion, the current study might reveal that the isolated bacteria could be of critical priority carbapenem-resistant *P. aeruginosa*, and carbapenem-resistant and 3rd generation cephalosporin-resistant *E. coli.* In addition, the isolation of high priority bacteria includes vancomycin-resistant methicillin-resistant *S. aureus*.

Keywords: Critical priority bacteria, Drug resistance profile, Extended-spectrum B-lactamase (ESBL), Hetero-resistance, High priority bacteria.

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INTRODUCTION

Infections caused by bacterial agents are responsible for high rates of morbidity and mortality worldwide.[1] Since 1928, when Fleming discovered penicillin, the modern era of antibiotics was started and antibiotics were introduced into clinical use. However, overuse of these antibiotics had led to the development of resistant bacterial populations.[2] The emergence of these bacteria is of significant medical concern and a serious global problem that results in recovery delay, a longer stay in hospitals, and an increase in public health costs.[3,4]

In recent years, the World Health Organization (WHO) has documented a list of antibiotic-resistant bacteria and classified them as critical, high, and medium priority.[5,6] Identification of the most common cause of bacterial infection in community and hospitals, and determine antibiotic susceptibility pattern for these isolated bacterial strains must be revised continuously to obtain a clear view about the current situation of antimicrobial resistance in order to improve treatment of infections and avoid using an unnecessary antibiotic that could enhance the development of bacterial resistance. Thus, the aim of this study is to identify the most common bacterial

**Author for Correspondence: shm.med.school@gmail.com*

agents responsible for infection and their antibiotic resistance rates.

MATERIALS AND METHODS

One hundred seventeen different bacteria isolated from different clinical samples, which were received during the period between September 2018 and February 2019, in Al-Kafeel Hospital in Kerbala governorate, were documented. Re-identification of the most common bacterial isolates was performed after transporting bacteria to the microbiology laboratory in the Department of Clinical Laboratories, College of Applied Medical Sciences, Kerbala University. The identification of microorganisms was conducted according to standard microbiological procedures.[7] Pure culture for isolates was obtained for susceptibility testing. Antibiotic susceptibility testing was performed by using the Kirby-Bauer disk diffusion method, and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[8] Mueller-Hinton agar (Himedia, India) was used with selected antibiotic discs. All antibiotics were purchased from Mast Diagnostic and Bioanalyse.

Statistical Analysis

Analysis was performed by using IBM Statistical Package for the Social Sciences (SPSS) 20.0 software. The frequency of resistance to each antibiotic was analyzed for the most common bacterial isolates.

Findings

Bacteriological laboratory procedures were applied in order to identify the bacterial causative agents for the received 117 different clinical samples. The results showed that *E. coli*, *S. aureus*, and *P. aeruginosa* represent the most common isolated bacteria with a percentage of 23.9, 18.8, and 16.2%, respectively.

Analyzing the correlation between the type of bacterial isolates and sex of the patients, revealed the presence of a significant correlation. Whereas, no significant correlation was found between the type of bacteria with patient admission status (data not shown).

After bacterial isolation, the antibiotic susceptibility pattern of the most common isolated bacteria was identified, as shown in Tables 1 to 3. *E. coli* strains were found to be highly resistant to amoxicillin-clavulanic acid, ceftriaxone (88.9%), ceftazidime (85.2%), trimethoprim-sulfamethoxazole (74.1%), ciprofloxacin (59.3%), and moderate resistance to levofloxacin (40.7%) and gentamycin (33.3%). Whereas, the highest sensitivity rates were seen with meropenem antibiotic (92.6%). In addition to that, 23 (~85%) of the isolated strains were resistant to both ceftriaxone and ceftazidime. Moreover, two isolates showed the presence of a hetero-resistance phenomenon (Figure 1). These two isolates were isolated from urine samples, and both were sensitive for meropenem and nitrofurantoin. The presence of hetero-resistance phenomena was approved by re-identification of the colonies that appeared in the inhibition zone by re-culturing on eosin methylene blue (EMB) agar (which is specific for *E. coli* by the appearance of green metallic sheen), and different biochemical test.

Concerning *S. aureus* isolates, 100 and approximately 91% of resistance rates were seen to penicillin and cefoxitin,

Table 1: Drug susceptibility pattern for *E. coli*

Antibiotic	*Sensitive*	*Intermediate*	*Resistant*	*Total*
Amoxi-clav	3 (11.1%)	0	24 (88.9%)	27
Ceftriaxone	3 (11.1)	0	24 (88.9%)	27
Ceftazidime	3 (11.1)	1 (3.7%)	23 (85.2%)	27
Ciprofloxacin	10 (37%)	1 (3.7%)	16 (59.3%)	27
Levofloxacin	13 (48.1%)	3 (11.1%)	11 (40.7%)	27
Gentamicin	15 (55.6%)	2 (7.4%)	9 (33.3%)	26
Amikacin	19 (70.4%)	4 (14.8%)	3 (11.1%)	26
Meropenem	25 (92.6%)	1 (3.7%)	1 (3.7%)	27
Trim-sulfa	6 (22.2%)	1 (3.7%)	20 (74.1%)	27

Table 2: Drug susceptibility pattern for *S. aureus*

Antibiotic	*Sensitive*	*Intermediate*	*Resistant*	*Total*
Penicillin	0 (0%)	0 (0%)	22 (100%)	22
Clindamycin	9 (40.9%)	1 (4.5%)	11 (50%)	21
Gentamycin	8 (36.4%)	2 (9.1%)	11 (50%)	21
Levofloxacin	15 (68.2%)	1 (4.5%)	6 (27.3%)	22
Trimethoprim-sulfamethoxazole	15 (68.2%)	0 (0%)	6 (27.3%)	21
Vancomycin	11 (50%)	1 (4.5%)	10 (45.5%)	22
Erythromycin	3 (13.6%)	0 (0%)	18 (81.8%)	21
Tetracycline	8 (36.4%)	0 (0%)	13 (59.1%)	21
Cefoxitin	0 (0%)	0 (0%)	20 (90.9%)	20

Table 3: Drug susceptibility pattern for *P. aeruginosa*

Antibiotic	*Sensitive*	*Intermediate*	*Resistant*	*Total*
Ceftazidime	12 (70.6)	1 (5.9)	4 (23.5)	17
Gentamycin	7 (41.2)	2 (11.8)	8 (47.1)	17
Amikacin	8 (47.1%)	1 (5.9%)	7 (41.2%)	16
Levofloxacin	9 (52.9%)	2 (11.8%)	6 (35.3%)	17
Ciprofloxacin	9 (52.9%)	0	8 (47.1%)	17
Meropenem	8 (47.1%)	2 (11.8%)	7 (41.2%)	17

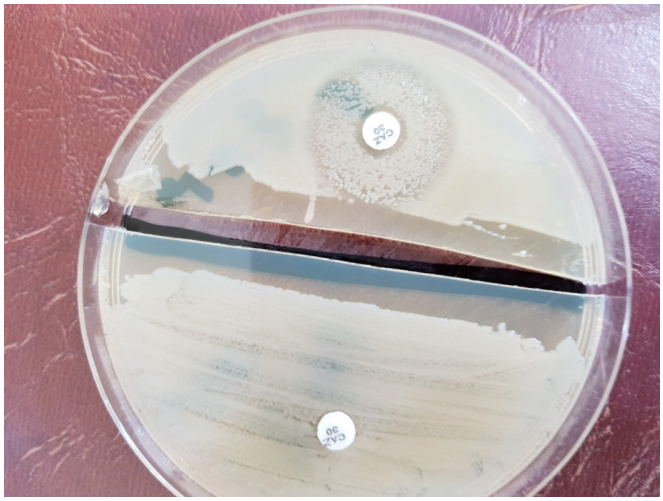

Figure 1: Hetero-resistance phenomenon; arrowhead shows *E. coli* isolate (isolated from a urine sample) with heteroresistance phenomena

respectively (MRSA). Approximately 50% of MRSA were VRSA, as shown in Table 2.

Moderate resistance rates were found with *P. aeruginosa* isolates to gentamycin and ciprofloxacin (47.1%), amikacin (41.2%), and levofloxacin (35.3%) (Table 3). *P. aeruginosa* isolates were found to be more resistant to meropenem than *E. coli* isolates, with a resistance rate of 41.2 and 3.7%, respectively.

DISCUSSION

Infections caused by antibiotic-resistant bacteria are considered as a threat worldwide because of the difficulties in their treatment and increase in cost thereof.[9] Thus, it is vital to continuously monitor the circulating bacteria, and their current status of antibiotic resistance.[10] A total of 117 different clinical samples were collected and bacteriological examination was performed. The results showed that *E. coli*, *S. aureus*, and *P. aeruginosa* represent the most common bacteria isolated with a percentage of 23.9, 18.8, and 16.2%, respectively. *E. coli* isolates represent the most common bacterial isolates predominate in urine samples (50%). Similar results were obtained from a previous study carried in Kerbala Province, Iraq.[11] On the other hand, a higher percentage of *E. coli* isolates (71%) predominating urinary tract infection was documented.[12-16]

100% of *P. aeruginosa* isolates and approximately 95% of *S. aureus* isolates were found in swab samples. This is in agreement with other previous studies, which documented the predominance of these two bacteria in swab samples.[17] In addition to that, the prevalence of these two pathogens (44.8 and 14.9%, respectively) were also documented in other studies.[18]

There was a significant correlation between the type of isolated bacteria and the sex of the patients. This result disagrees with other studies.[16,18] However, there were a lot of studies that documented the effect of sex hormones on the immune system and propensity to infectious agents.[19,20] Testosterone has an immunosuppressive effect and estrogens can enhance it, thus, male patients were more prone to infection. The current study showed that bacterial infection mainly occurs in men, which may be due to sex hormones that make men more susceptible to infection.

Additionally, no significant correlation was found in the type of bacteria with patient admission status. This may reflect that the same bacterial agents were circulating in the community and hospitals, and account for most common infections in the Province.

Antibiotic susceptibility patterns of *E. coli* isolates were found to be highly resistant to amoxicillin-clavulanic, ceftriaxone, ceftazidime, trimethoprim-sulfamethoxazole, ciprofloxacin, and moderate resistance to levofloxacin and gentamycin. Whereas, the highest sensitivity rates were seen to meropenem antibiotic. The presence of high resistant rates could be attributed to the consumption of antibiotics in a certain area, and a certain association was found between self-use of certain antibiotic and resistance to it,[21] for example, the broad use of amoxicillin-clavulanic acid for urinary and respiratory tract infection resulted in very high resistance rates to it.[22]

In addition to that, 23 (~85%) of the isolated strains were resistant to both ceftriaxone and ceftazidime. According to CLSI, if the tested isolate showed resistance for both ceftriaxone and ceftazidime, therefore, this isolate must be tested for the presence of ESBL enzymes. ESBL enzymes are plasmid-mediated β-lactamases that inhibit extended-spectrum cephalosporins, monobactam, and aztreonam, and were detected first in *Enterobacteriaceae*, and then detected in other microorganisms, and are increasingly reported worldwide thereafter. Therefore, these enzymes can limit the effect of chemotherapeutic agents, and infection control and management.[23-25]

Moreover, the current study revealed that two isolates of *E. coli* showed the presence of hetero-resistance phenomenon to ceftazidime and gentamicin. Hetero-resistance is defined as the presence of a subpopulation of the same bacterial isolate (genetically, identical), showing a range of resistance

to a certain antibiotic. This phenomenon was first described in 1947 for *Haemophilus influenza*, and then for gram-positive bacteria.[27] The emergence of these isolates in the community may be attributed to the overuse of antibiotics that leads to the adaptation of bacteria to the antibiotic, which may result in treatment failure.[26,27]

Concerning *S. aureus* isolates, all of the isolates were resistant to penicillin, and approximately 91% of them were resistant to cefoxitin (MRSA). Approximately 50% of MRSA were VRSA. The prevalence of MRSA in Kerbala Province is in continuous increase, which is documented by many previous studies.[28,29]

Moderate resistance rates were found with *P. aeruginosa* isolates to gentamycin, ciprofloxacin, amikacin, and levofloxacin (Table 5). *Pseudomonas* isolates were found to be more resistant to meropenem than *E. coli* isolates, with a resistance rate of 41.2 and 3.7%, respectively. *P. aeruginosa* is one of the microorganisms with limited therapeutic options due to resistance to several classes of antibiotics that come from different mechanisms, like plasmid-associated resistance to different classes of antibiotics.[30-33]

CONCLUSION

The results of the current study showed that *E. coli*, *S. aureus*, and *P. aeruginosa* represent the most common bacteria isolated with high resistance rates to different classes of antibiotics and that bacterial infection is mainly occurring in men. The current study revealed the isolation of critical priority carbapenem-resistant *P. aeruginosa*, and carbapenem-resistant and 3rd generation cephalosporin-resistant *E. coli.* In addition, isolation of high priority bacteria includes vancomycin-resistant and methicillin-resistant *S. aureus*. No significant correlation was observed in the type of isolated bacteria with patient admission status. Other important findings were the possibility of the presence of ESBLs producing bacteria, and the appearance of hetero-resistance phenomenon.

ETHICAL CLEARANCE

No approval from the ethics committee was required, as the testing was performed on residual bacterial samples, and the results were not used for the patient's therapeutic management.

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