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Antimicrobial Susceptibility Pattern of Six Threatening Pathogens at Mofid Children's Hospital, Tehran, Iran

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Abstract

Background: The treatment of bacterial infections is increasingly complicated due to the ability of bacteria to develop resistance to antimicrobial agents. The aim of this study was to survey the antimicrobial susceptibility patterns of several pathogens isolated from in- and out-patients at Mofid children's hospital.

Methods: From October 2015 to April 2016, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus* spp. detected from clinical (urine and non-urine) specimens of inpatient and outpatient were survived. Sensitivity was measured by disc diffusion method based on the CLSI recommendation.

Results: Totally, *E. coli* (62.7%) and *P. aeruginosa* (42.3%) were the predominantly isolated pathogens in this study from urine culture and non-urine culture, respectively. All cultured staphylococcal isolates were susceptible to vancomycin. The most effective antibiotics for Gram-negative bacteria were meropenem, amikacin, and imipenem, in sequence. None of the Gram-negative bacteria was sensitive to tetracycline.

Conclusions: Our findings showed that there was a considerable geographic variation in bacterial patterns and antibiotic susceptibility properties. Therefore, monitoring of antibiotic sensitivity pattern is helpful for selecting antibiotics for empiric therapy.

Keywords: Microbial Sensitivity Test, Inpatient, Outpatient, Pathogens

1. Background

The emergence of resistance to antimicrobial agents in hospitals and in the community has become an important public health concern (1-4). Each year in the United States, at least 2 million people acquire serious infections with bacteria that are not susceptible to one or more of the antibiotics designed to treat those infections. At least, 23,000 people die each year as a direct result of these antibiotic-resistant infections. Much more also die from other conditions that were complicated by antibiotic-resistant infections (5). The emergence of resistance to an antimicrobial agent is associated with increased morbidity and mortality (6-9). The importance of decreased sensitivity to available antibiotics is a growing concern in hospitals due to increased rates of multi-drug resistant (MDR) pathogens in Iranian healthcare facilities (9-12). Since there are a few data regarding the bacterial susceptibility pattern in Iran, it is essential to prospectively evaluate the distribution of bacterial species isolated and their susceptibility pattern, especially in the case of threatening bacteria.

In the present study, we aimed to identify the antimicrobial susceptibility pattern of Escherichia coli, Pseudomonas aeruginosa, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus* spp. among hospitalized patients and outpatients to provide a feasible guide for clinicians.

2. Methods

2.1. Study Area and Bacterial Identification

This retrospective cross-sectional study was conducted within six months from October 2015 to April 2016. We surveyed antimicrobial sensitivity of *E. coli*, *P. aeruginosa*, *Acinetobacter* spp., *K. pneumoniae*, *S. aureus*, and *Enterococcus* spp., which were detected from urine and non-urine sites (wound, CSF, blood, sputum, trachea, eye, and nose discharge) in the laboratory of Mofid children's hospital affiliated to Shahid Beheshti University of Medical Sciences. Inpatients and outpatients were included. Mofid Hospital is a referral, tertiary care center that contains 300 beds and different wards in Tehran, the capital of Iran.

2.2. Antimicrobial Susceptibility Testing

The susceptibility profile was determined by following locally available antibiotics by using the disk (Mast, UK) diffusion method in accordance with the CLSI recommendation (13), and the test was performed on Mueller-Hinton agar (Merck, Germany). The antimicrobial disks were as follows for Gram-positive isolates: penicillin (10 units), ampicillin (10 μg), vancomycin (30 μg), co-trimoxazole (25 μg), chloramphenicol (30 μg), gentamicin (10 μg), ciprofloxacin (5 μg), oxacillin (1 μg), azithromycin (15 ug), linezolid (10 μg), tetracycline (30 μg), doxycycline (30 μg), rifampicin (5 μg), and clindamycin (2 μg) and for Gram-negative isolates: ampicillin (10μg), amikacin (30 μg) tobramycin (10 μg), piperacillin (30 μg), amoxicillin-clavulanic acid (20 – 10 μg), ampicillin-sulbactam (10 - 10μg), cefuroxime (30 μg), cefepime (30 μg), levofloxacin (5 μg), ticarcillin-clavulanic acid (75 - 10μg), meropenem(10 μg), imipenem (10 μg), ceftazidime (30 μg), co-trimoxazole (25 μg), chloramphenicol (30 μg), gentamycin (10 μg), ciprofloxacin (5 μg), tetracycline (30 μg), doxycycline (30 μg), cephazolin (30 μg), and piperacillin-tazobactam (100 – 10 μg). The plates were incubated aerobically at 37°C for 18 hours and the interpretation of the results of the antimicrobial susceptibility was made based on the clinical and laboratory standards institute (CLSI) criteria. In our results, intermediate isolates considered as resistant. *S. aureus* ATCC 29213, *E. coli* ATCC25922, and *P. aeruginosa* ATCC 27853 were used as the standard quality controls. MDR isolates were estimated according to previously described definitions (14).

2.3. Statistical Analysis

The analysis was performed by using SPSS^{TM} software, version 21.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency. Values were expressed as the percentages in every group (for categorical variables).

3. Results

During the six-month period of this study, 867 pathogens were detected; we presented the result based on urine and non-urine site.

3.1. Urine Culture

Among 467 pathogens isolated from urine cultures, 92 (19.7%) Gram-positive bacteria and 375 (80.3%) Gram-negative bacteria were detected.

The most common bacteria were *E. coli* (293, 62.74%), followed by *Enterococcus* spp. (73, 15.63%), *Klebsiella* spp. (63, 13.5%), *S. aureus* (19, 4.06%), *Pseudomonas* spp. (17, 3.64%), and *Acinetobacter* spp. (2, 0.43%). In urine cultures, the majority of samples were gathered from outpatients and the nephrology ward with 60.8% and 13.5%, respectively. Tables 1 and 2 demonstrate the frequency of antimicrobial susceptibility of the important pathogens isolated from urine cultures. For Gram-negative bacteria, the susceptibility pattern of antibiotics showed that imipenem, meropenem, and amikacin were most effective antibiotics and ciprofloxacin and ceftazidime showed an acceptable sensitivity. In addition, for Gram-positive bacteria, vancomycin and chloramphenicol showed 100% sensitivity for staphylococci and 47.9 and 89.7% for enterococci isolates, respectively.

Table 1. The Antibiotic Susceptibility Pattern of Gram-Positive Isolates Recovered From The Urine Culture[a]

Gram-Positive Isolates	*S. aureus* (N = 19)	*Enterococcus* spp.(N = 73)
Penicillin	3 (15.8)	15 (20.5)
Ampicillin	3 (15.8)	28 (38.4)
Vancomycin	19 (100)	35 (47.9)
Linezolid	0 (0)	0 (0)
Tetracycline	0 (0)	0 (0)
Oxacillin	5 (26.3)	0 (0)
Azithromycin	0 (0)	0 (0)
Clindamycin	0 (0)	1 (1.4)
Trimethoprim-sulfamethoxazole	6 (31.6)	2 (2.7)
Doxycycline	0 (0)	0 (0)
Rifampicin	0 (0)	0 (0)
Gentamicin	5 (26.3)	20 (27.4)
Ciprofloxacin	13 (68.4)	14 (19.2)
Chloramphenicol	19 (100)	65 (89)

[a]Values are presented as No. (%).

3.2. Non-Urine Culture

Out of 400 pathogens isolated from non-urine clinical samples, 97 (24.2%) Gram-positive bacteria and 303 (75.8%) Gram-negative bacteria were detected. The predominant isolates were *Pseudomonas* spp. (169, 42.25%), followed by *S. aureus* (88, 22%), *Klebsiella* spp. (65, 16.25%), *E. coli* (45, 11.25%), *Acinetobacter* spp. (24, 6%), and *Enterococcus* spp. (9, 2.25%). The majority of these samples were obtained from pediatric intensive care unit (PICU) and outpatients as 19.5% and 18%, respectively.

The antimicrobial susceptibility pattern of the isolated pathogens from non-urine samples is shown in Tables 3

Table 2. The Antibiotic Susceptibility Pattern of Gram-Negative Isolates Recovered From The Urine Culture[a]

Gram-Negative Isolates	*E. coli* (N = 293)	*Klebsiella* spp. (N = 63)	*Acinetobacter* spp. (N = 2)	*Pseudomonas* spp. (N = 17)
Ampicillin	20 (6.8)	6 (9.5)	0 (0)	0 (0)
Cephazolin	31 (10.6)	7 (11.1)	0 (0)	0 (0)
Gentamicin	167 (57)	34 (54)	0 (0)	10 (58.8)
Tobramycin	43 (14.7)	6 (9.5)	0(0)	3 (17.6)
Piperacillin	18 (6.1)	3 (4.8)	1 (50)	7 (41.2)
Amoxicillin clavulanic acid	0 (0)	0 (0)	0 (0)	0 (0)
Ampicillin sulbactam	40 (13.7)	9 (14.3)	0 (0)	0 (0)
Piperacillin-tazobactam	0 (0)	0 (0)	0 (0)	0 (0)
Cefepime	11 (3.8)	4 (6.3)	0 (0)	1 (5.9)
Cefotaxime	27 (9.2)	3 (4.8)	0 (0)	0 (0)
Cefuroxime	10 (3.4)	3 (4.8)	0 (0)	1 (5.9)
Imipenem	229 (78.2)	47 (74.6)	0 (0)	12 (70.6)
Meropenem	277 (94.5)	58 (92.1)	1 (50)	15 (88.2)
Amikacin	273 (93.2)	47 (74.6)	1 (50)	14 (82.4)
Ciprofloxacin	186 (63.5)	42 (66.7)	0 (0)	11 (64.7)
Trimetoprim-sulfametoxazol	33 (11.3)	11 (17.5)	0 (0)	0 (0)
Ceftazidime	148 (50.5)	28 (44.4)	1 (50)	16 (94.1)
Ticarcillin-clavulanic acid	0 (0)	0 (0)	0 (0)	0 (0)
Tetracycline	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	1 (3)	0 (0)	0 (0)	1 (5.9)
Levofloxacin	0 (0)	0 (0)	0 (0)	0 (0)
Doxycycline	0 (0)	0 (0)	0 (0)	0 (0)

[a]Values are presented as No. (%).

and 4. *Enterococcus* spp. were most susceptible to chloramphenicol (88.9%) and vancomycin (33.3%). *S. aureus* isolates were sensitive to vancomycin and chloramphenicol as 100% and 78.4%, respectively. Most effective antibiotics for *Pseudomonas* spp. were ciprofloxacin (81.1%) and ceftazidime (70.4%). *E. coli* and *Klebsiella* spp. showed high sensitivity rates to meropenem, imipenem, and amikacin. In overall, the most MDR isolates were enterococci and *Acinetobacter* spp. both with 100% prevalence (Table 5).

Table 3. The antibiotic susceptibility of Gram-Positive Isolates Recovered From The Non-Urine Culture[a]

Gram-Positive Isolates	*S. aureus* (N = 88)	*Enterococcus* spp. (No = 9)
Penicillin	2 (2.3)	0 (0)
Ampicillin	5 (5.7)	0 (0)
Vancomycin	88 (100)	3 (33.3)
Linezolid	0 (0)	0 (0)
Tetracycline	0 (0)	0 (0)
Oxacillin	21 (23.9)	0 (0)
Azithromycin	2 (2.3)	0 (0)
Clindamycin	1 (1.1)	0 (0)
Trimethoprim-sulfamethoxazole	20 (22.7)	0 (0)
Doxycycline	0 (0)	0 (0)
Rifampicin	3 (3.4)	0 (0)
Gentamicin	4 (4.5)	0 (0)
Ciprofloxacin	39 (44.3)	0 (0)
Chloramphenicol	69 (78.4)	8 (88.9)

[a]Values are presented as No. (%).

4. Discussion

Consistent with our results, the most common nosocomial pathogens in Ott et al. study were *E. coli* and *S. aureus*. Moreover, the most common pathogens isolated in Azim et al. study from India were *P. aeruginosa* and *A. baumannii* (15). In Aly and Balkhy study from six Arab countries including Saudi Arabia, Qatar, Bahrain, Kuwait, Oman, and United Arab Emirates, the most prevalent determined microorganisms were *E. coli*, *K. pneumoniae*, *P. aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Acinetobacter* spp. (16). In our results, *E. coli* (62.7%) was the predominant isolated pathogen in urine cultures. The role of *E. coli* as the predominant causative agent of urinary tract infections (UTIs) was confirmed in most of the previous studies (17-23). In our findings, S. aureus and enterococci were seen in small numbers, but they were recognized as important causes of UTIs (24-26). The general distribution pattern of our study showed the most com-

Table 4. The Antibiotic Susceptibility of Gram-Negative Isolates Recovered From The Non-Urine Culture[a]

Gram -Negative Isolates	*E. coli* (N = 45)	*Klebsiella* spp. (No = 65)	*Acinetobacter* spp. (N = 24)	*Pseudomonas* spp. (N = 169)
Ampicillin	3 (6.7)	2 (3.1)	0 (0)	2 (1.2)
Cephazolin	0 (0)	0 (0)	0 (0)	0 (0)
Gentamicin	15 (33.3)	13 (20)	1 (4.2)	36 (21.3)
Tobramycin	2 (4.4)	2 (3.1)	4 (16.7)	27 (16)
Piperacillin	0 (0)	6 (9.2)	3 (12.5)	49 (29)
Amoxicillin clavulanic acid	0 (0)	0 (0)	0 (0)	0 (0)
Ampicillin sulbactam	2 (4.4)	3 (4.6)	0 (0)	0 (0)
Piperacillin-tazobactam	0 (0)	0 (0)	0 (0)	2 (1.2)
Cefepime	11 (24.4)	8 (12.3)	0 (0)	31 (18.3)
Cefotaxime	1 (2.2)	0 (0)	0 (0)	4 (2.4)
Cefuroxime	7 (15.6)	8 (12.3)	2 (8.3)	32 (18.9)
Imipenem	31 (68.9)	49 (75.4)	5 (20.8)	96 (56.8)
Meropenem	43 (95.6)	56 (86.2)	5 (20.8)	113 (66.9)
Amikacin	27 (60)	28 (43.1)	8 (33.3)	89 (52.7)
Ciprofloxacin	25 (55.6)	49 (75.4)	13 (54.2)	137 (81.1)
Trimetoprim-sulfametoxazol	1 (2.2)	5 (7.7)	1 (4.2)	7 (4.1)
Ceftazidime	23 (51.1)	26 (40)	6 (25)	119 (70.4)
Ticarcillin-clavulanic acid	0 (0)	0 (0)	0 (0)	0 (0)
Tetracycline	0 (0)	0 (0)	0 (0)	0 (0)
Chloramphenicol	1 (2.2)	0 (0)	2 (8.3)	8 (4.7)
Levofloxacin	0 (0)	0 (0)	0 (0)	0 (0)
Doxycycline	0 (0)	0 (0)	0 (0)	0 (0)

[a]Values are presented as No. (%).

Table 5. The Distribution of MDR Isolates From Urine And Non-Urine Culture[a]

Isolates	*S. aureus*	*Enterococcus* spp.	*E. coli*	*Klebsiella* spp.	*Acinetobacter* spp.	*Pseudomonas* spp.
Urine culture	17 (89.4)	67 (91.8)	250 (85.3)	57 (90.5)	2 (100)	16 (94.1)
Non-urine culture	83 (94.3)	9 (100)	36 (80)	57 (87.7)	22 (91.6)	157 (92.9)

[a]Values are presented as No. (%).

monly isolated bacteria from non-urine samples were *P. aeruginosa* (42.3%). Previously, some other studies showed the same results including Mukherjee et al. as 61, Japoni et al. as 67.7%, and Karlowskyet et al. as 42%, and Ghassemi et al. observed *P. aeruginosa* as the most common pathogen in their findings (23, 27, 28). However, it could be due to nosocomial infection or contamination of samples with this organism.

The surveillance of bacterial antimicrobial resistance is the most important challenge to understanding the dynamics of decreasing susceptibility to antibiotics in both hospital and community-acquired pathogens (29). The highest antibiotic susceptibility rate of *E. coli* and *Klebsiella* isolates obtained from urine and non-urine cultures was to meropenem, followed by amikacin, imipenem, and ciprofloxacin, in sequence. Overall, the most effective antibiotics against Gram-negative bacteria were meropenem, amikacin, and imipenem with approximately 50 - 95% susceptibility.

The rates of imipenem susceptibility in our study were different from those reported in previous studies showing that the isolates of *Klebsiella* spp. and *E. coli* were fully susceptible to imipenem (30, 31). The cause of these differences may be due to higher carbapenem prescription for patients in our study hospital and subsequently the emergence of carbapenem-resistant strains.

In our study, the susceptibility rate of *P. aeruginosa* to imipenem was lower than the susceptibility rate reported by Mohammadi et al. from the west of Iran (32). All of the *S. aureus* isolates in our study showed 100% susceptibility to vancomycin. This pattern is the same as the findings of other Iranian studies (22, 33-36).

The high rate of sensitivity to vancomycin for Gram-positive cocci was noted in several previous studies from Iran and other parts of the world (18, 21). On the other hand, in our study, meropenem, amikacin, and imipenem are relatively effective drugs for the treatment of the majority of the infections caused by Gram-negative bacterial isolates,

especially Enterobacteriaceae. This can be due to that these antibiotics may not be commonly used before and/or they are newly introduced. These findings were in accordance with the previous results from different parts of the world (32, 34, 37), but Armin et al. presented contrary results (38) that could be due to differences in the population study. The emergence of MDR strains is increasing the health concern. In the present study, we found a remarkable rate of MDR isolates. Previously, in accordance with our findings, several reports showed the increasing prevalence of MDR strains in Iranian hospitals (11, 39-42).

4.1. Conclusion

Considerable differences in antimicrobial resistance do exist for every single hospital that may reflect differences in antimicrobial usage pattern, population study, and infection control strategies. In this study, Gram-positive bacteria showed a high rate of susceptibility to chloramphenicol, which recently was rarely prescribed by our physicians. Gram-negative bacteria showed the most susceptibility to amikacin, meropenem, and imipenem, in sequence.

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