Frequency of bacterial isolates and antibiotics resistance patterns in urine and pus samples.

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ABSTRACT… Objectives: The present study is aimed to determine frequency of bacteria in urine and pus sample along with the antibiotic resistance profile of isolated bacteria. **Study Design:** Cross-Sectional Study. **Setting:** Real-Time PCR Laboratory at Dabgari Garden, Peshawar with the Collaboration of Health Biotechnology Laboratory, Department of Biotechnology, Abdul Wali Khan University, Mardan. **Period:** June 2018 to July 2019. **Material & Methods:** The urine and pus samples were collected from suspected patients and were cultured on appropriate culture media. The biochemical tests were also performed after growing on culture plates for identification and confirmation of bacterial isolates. The disc diffusion technique was used to evaluate the antibiotic pattern of retrieved isolates. **Result:** A total of 525 samples of pus and urine samples were collected from different regions of Peshawar. Out of the total, 237 (45.1%) samples were found positive for bacterial growth whereas the remaining (54.9%) were observed negative. Among total positive isolates, 220 (92.8%) were from urine samples, and 17 (7.2%) were from pus samples. The predominant isolate was Escherichia coli (E. coli) (90.3%) retrieved from positive samples, followed by Pseudomonas aeruginosa (7.2%), Klebsiella pneumonia (1.3%), and Staphylococcus aureus (1.3%). The most effective antibiotic was Fosfomycin against bacteria whereas Nalidixic acid, Nitrofurantoin, and Amoxicillin/clavulanic acid were found less effective against bacterial isolates. **Conclusion:** Most frequent bacteria isolated was E. coli and the most efficient drug was Fosmomycin and the least was Nitrofurantoin, and Amoxicillin.

Key words: Antibiotics, E. Coli, Peshawar, Pus, Uropathogens.

INTRODUCTION

Antibacterial drugs are necessary for the protection of public health from bacterial infections. However, antibiotic resistance is becoming a challenge for public health and health professionals.[1] The high proportion of bacterial infection including bloodstream infection along with other related diseases needs special attention.[2] The frequency of bacterial infection caused by resistant bacterial isolates were more than 50%.[3] From the last few decades, antimicrobial-resistant is increasing continuously.[4] The management of bacterial infection is critically associated with specific and appropriate antibiotics. The bacterial infections caused by resistant isolates are the leading public health issue. Many categories of drugs currently exist for the treatment and management of bacterial infections including Aminoglycosides, Cephalosporin (various classes), Fluoroquinolones, Macrolides, Penicillin, and Tetracycline.[5]

The predominant isolates of urine and pus are highly resistant to antibiotics such as Carbapenem.[6] Various mechanisms are related to bacterial resistance against antibiotics are known including enzyme-producing bacteria (Metallo beta-lactamases and extended-spectrum beta-lactamases).[7] The mechanism of resistance to carbapenems such as efflux pumps, producing carbapenemase, and porins actions are frequently associated.[8] Mostly gram-negative bacterial isolates show resistance to antibiotics due to enzymes.[9,10]

The Enterobacteriaceae family is primarily involved in bacterial infection particularly in urinary tract infections. The common resistance caused by Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumoniae), Pseudomonas aeruginosa (P. aeruginosa), and Staphylococcus aureus (S. aureus) related to antibiotics due to misuse of antibiotics, an overdose of drugs, non-adherence, and non-compliance.[11,12] The purpose of the study was to find out bacterial isolates in urine and pus samples and the most efficient drug used against these bacterial isolates.

MATERIAL & METHODS
The cross-sectional study was conducted in Real-Time PCR Laboratory at Dabgari Garden, Peshawar with the Collaboration of Health Biotechnology Laboratory, Department of Biotechnology, Abdul Wali Khan University, Mardan. The study duration was from June 2018 to July 2019. A total of 525 samples were collected. Simple conveient technique was used for sample collection.

Inclusion Criteria
All volunteer patients were included irrespective of age and gender.

Exclusion Criteria
Non-volunteer and samples other than urine and pus were excluded.

The collected samples of pus and urine were transported to the laboratory and were cultured on their specific media such as MacConkey agar, Blood agar, and Cysteine Lactose Electrolyte Deficient (CLED) agar. The bacterial growth was determined after 18-24 hours. The bacterial isolates were further processed through biochemical tests for identification and confirmation of gram-positive and gram-negative bacterial isolates. The gram staining procedure was applied for the identification of bacteria and was examined under the 100X lens of microscope.[13,14]

The morphological colonies of E. coli, P. aeruginosa, K. pneumoniae, and S. aureus were identified as per the recommended protocol.[15] The disc diffusion method was used for antibiotics susceptibility testing. The Muller Hinton Agar was used for the disc diffusion technique.[16] Several locally available antibiotics discs were used including Amikacin (30µg), Ampicillin (10µg), Amoxicillin-clavulanic acid (20/10µg), Ceftazidime (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Clindamycin (2µg), Cotrimoxazole (1.25µg), Gentamicin (10µg), Imipenem (10µg), Methicillin (5ug), Nalidixic Acid (30), Nitrofurantoin (300µg), Oxacillin (1µg), and Vancomycin (30µg).[17]

Microsoft Excel 2007 was used for the initial analysis of data, which was further analyzed by statistical package for social science version 22. Ethical approval for study was obtained from Abdul Wali Khan University, Mardan (AWKUM/Biotech/2018/806).

RESULTS
A total of 525 samples were collected from patients irrespective of age, gender, and ethnicity. Out of total, 88.0% (n=462) were urine samples, while 12.0% (n=63) were pus samples. Among the total samples (n=525), 45.1% (n=237) were found positive and showed bacterial growth while remaining were negative. Among the positive culture, 92.8% (n=220) and 7.2% (n=17) were observed in urine and pus samples respectively (Table-I).

Samples	Culture	Percentage (Frequency) % (n)
Urine Sample (n=462)	Positive	47.6 (220)
	Negative	52.4 (242)
Pus Sample (n=63)	Positive	27.0 (17)
	Negative	73.0 (46)
Total	Positive	45.1 (237)
	Negative	44.9 (288)

Table-I. Frequency of culture-positive and negative in urine and pus samples.

The frequency of bacterial isolates was also evaluated in both pus and urine specimens of patients. The proportion of bacterial isolates including E. coli, P. aeruginosa, K. pneumoniae, and S. aureus was 90.3%, 7.2%, 1.3%, and 1.3% respectively, in urine and pus specimens. The most

common uropathogen was E. coli, determined in 93.6% (n=206) urine samples, followed by P. aeruginosa (5%), and K. pneumoniae (1.4%). Similarly, E. coli was also predominantly ascertained in 47.1% pus samples, followed by P. aeruginosa (35.3%) and S. aureus (17.6%) (Table-II).

The antibiotic susceptibility patterns of different isolates were determined, using available antibiotics. The E. coli showed the highest resistance to Nalidixic acid (83%), followed by Nitrofurantoin (75%), Norfloxacin (75%), Cefotaxime, Ceftriaxone, Ciprofloxacin and Ceftazidime (67% each). However, Meropenem, Amikacin, and Fosfomycin and Piperacillin/ Tazobactam were the most effective antibiotic against E. coli with a proportion of 99%, 99%, 98% and 96% respectively (Table-III).

P. aeruginosa showed high resistance to Nitrofurantoin (100%), followed by Amoxicillin/ Clavulanic Acid (99%), Nalidixic acid (81%), and Ceftriaxone (71%). Whereas, highly susceptible to Amikacin (100%), Meropenem (100%), and Tazobactam/Pipercillin (100%), Fosfomycin (76%), Ceftazidime (64%) and Ciprofloxacin (59%) (Table-III).

The K. pneumoniae were highly resistant to Nitrofurantoin (100%), followed by Cefotaxime, Piperacillin, Nalidixic acid, Ceftriaxone, and Ciprofloxacin. The most effective antibiotics were Meropenem, Aztreonam, Fosfomycin, Amikacin, Norfloxacin, and Piperacillin/ Tazobactam (Table-III).

S. aureus was predominantly resistant to Aztreonam (100%), Nalidixic acid (100%), Piperacillin (100%), and Amoxicillin/Clavulanic Acid (100%). Whereas, the highly effective antibiotics were fosfomycin having 0% resistance, followed by Ceftriaxone, Cefotaxime, Ceftazidime, and Amikacin (Table-III).

Sr. No.	E. coli % (n)	P. aeruginosa % (n)	K. pneumoniae % (n)	S. aureus % (n)	Total % (n)
Urine	93.6 (206)	5.0 (11)	1.4 (03)	0 (0)	92.8 (220)
Pus	47.1 (08)	35.3 (06)	0 (0)	17.6 (03)	7.2 (17)
Total	90.3 (214)	7.2 (17)	1.3 (03)	1.3 (03)	100 (237)

Table-II. Comparative occurence of bacteria (E. coli, P. aeruginosa, K. pneumoniae, and S. aureus) in both urine and pus samples.

Sr. No.	Antibiotics	E. Coli %	P. aeruginosa %	K. pneumonia %	S. aureus %
1	Aztreonam	62	53	33	100
2	Fosfomycin	2	24	33	0
3	Cefotaxime	67	56	67	33
4	Ceftriaxone	67	71	67	0
5	Ciprofloxacin	67	41	67	67
6	Ceftazidime	67	59	-	33
7	Meropenem	1	0	0	67
8	Amikacin	1	0	33	33
9	Nalidixic Acid	83	81	67	100
10	Norfloxacin	75	56	33	-
11	Piperacillin/ Tazobactam	4	0	33	67
12	Piperacillin	NT	NT	67	100
13	Nitrofurantoin	75	100	100	-
14	Ceftizoxime	12	53	-	100
15	Amoxicillin/ Clavulanic Acid	55	99	33	67

Table-III. Antibiotic-resistant pattern of E. coli, P. aeruginosa, K. pneumoniae and S. aureus collected from urine and pus samples.

DISCUSSION

Frequency and pattern of antibiotic-resistant were determined in the present study. The results of the study show that the culture-positive proportion was found in 45.1% samples both in urine and pus. The proportion of gram-negative bacteria was more than gram-positive bacteria in the present study. Similar to the current study, a study revealed from China conducted during 2015-2018, which shows a 43% prevalence of bacterial isolates in clinical samples, in which gram-negative bacterial isolates was 88%.[18,19] The result of the present study is also in accordance with the other study carried out in Pakistan.[20]

This study results also revealed that the percentage of E. coli, P. aeruginosa, K. pneumoniae, and S. aureus was 90.3%, 7.2%, 1.3%, and 1.3% respectively. In the earlier studies, a similar report revealed that the growth of E. coli, P. aeruginosa, K. pneumoniae, and S. aureus were observed in 73%, 7%, 1.5%, and 1%, respectively.[21] Our results are in accordance with a previous study in which infections due to E. coli was 90% while other microorganisms causing UTI were only 10%. The rate of resistance was 50.6% while 45% culture positivity which is similar to the finding of a study carried in China.[22]

The study carried out in 2015, which shows that the resistance of E. coli against Amikacin, Amoxicillin/ Clavulanic acid, Ampicillin, and Aztreonam was 91%, 84%, 84%, and 72% respectively.[23]

The present study shows that E. coli was highly resistant to eight different antibiotics including Nalidixic acid, Nitrofurantoin, and Norfloxacin. Similar to other studies conducted by various researchers in a different region of the world showed the highest susceptibility to Fosfomycin, Imipenem, Meropenem, and Piperacillin/ Tazobactam.[24]

In the current study, the sensitivity of E. coli was Amikacin, Meropenem, Fosfomycin, and Piperacillin/Tazobactam was 99%, 99%, 98%, and 96% respectively. P. aeruginosa was found the second most common gram-negative bacterial isolates in urine and pus samples, which shows frequent resistance to Nitrofurantoin, Amoxicillin/ Clavulanic acid, Nalidixic acid, and Ceftriaxone. On the other hand, most efficient antibiotics were Amikacin, Meropenem, and Tazobactam/ Piperacillin against the second most prevalent bacteria. K. pneumoniae showed less resistance as compared to P. aeruginosa. The Meropenem and Amikacin were found most effective antibiotic against K. pneumoniae with 0% (zero) resistance, while found less efficient antibiotics were Nitrofurantoin, Nalidixic acid, and Ceftriaxone. In the previous studies, the proportion of resistance was high against Norfloxacin and Ciprofloxacin.[25] A study also revealed a 100% sensitivity of Nitrofurantoin against K. pneumonia.[16] The S. aureus was found only gram-positive bacterial isolate in the present study, which is 100% resistant to Aztreonam, Ceftizoxime, Nalidixic acid, and Piperacillin. However, Fosfomycin and Ceftriaxone was 100% effective against S. aureus. These findings of the present were consistent with previously conducted studies, which revealed that Amikacin is a highly sensitive antibiotic.[26] On the other side, the resistance of S. aureus was noted against Ampicillin, Amoxicillin, Ciprofloxacin, Levofloxacin, and Norfloxacin.[27] Another study revealed that Nitrofurantoin was the most efficient antibiotic.[28] According to previous reports, the resistance against antibiotics from 2005 to 2014 was similar to the present study.[29] A recent study in China conducted showed that the resistance proportion was consistent with both gram-negative bacteria and gram-positive bacteria.[30,31] A study showed 50% resistance to P. aeruginosa for a wide range of antibiotics.[32] In a past study conducted in India observed that the Amikacin (99.6%), Nitrofurantoin (91%), and Ciprofloxacin (88%) was susceptible[33] which is similar to the results of Amikacin but discord with results the Ciprofloxacin and Nitrofurantoin, which was 0% (zero) in the study.

A few limitations were also found with current study, molecular determination was not study of isolated isolates, clinical demographic data and samples other than urine and pus were not studied.

CONCLUSION

In summary, the frequency of E. coli infection was greater than the rest of the gram-negative isolates. In the gram-positive isolates, S. aureus was found only bacterial isolate. Overall, the rate of growth of gram-positive was 1.3% whereas the gram-negative strains were 98.7%. The antibiotics resistance pattern was also observed, where E. coli, P. aeruginosa and K.pneumoneae were susceptible 100% to Amikacin and Meropenem along with the Piperacillin/Tazobactam against all three gram-negative species. Fosfomycin was most effective against the retrieved bacteria. Based on the current finding, proper management is essential to overcome the resistance of antibiotics. The periodic surveillance of antibiotics is highly recommended to determine the pattern of resistance of antibiotics.

Conflict of Interest
Authors have no conflict of interest.
**Copyright© 08 Feb, 2021.**

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4	Syed Luqman Shuaib	Critical studies, Conceptualization of study, manuscript writeup, review and approval.	
5	Anees Muhammad	Literature review, data entry, data analysis, manuscript writeup, review and approval.	