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Original Article

Increasing Antibiotic Resistant Pattern in Clinical Bacterial Isolates, From Tertiary Care Hospital, Hayatabad Medical Complex, Peshawar, Pakistan

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ABSTRACT

Bacterial infections are spreading worldwide especially in the developing countries. Most clinical pathogens have evolved mechanismsof resistance due to which most antibiotics are less or not effective to restrict their growth. **Objective:** To find the prevalence of antibiotics resistance in clinical isolates. M**ethods:** Total (n=753) clinical specimens were collected, among them, total (n=105) bacteria were identified on the basis of standard culture characteristics and biochemical tests and their antibiotics resistance pattern were determined **Results:** Higher incidence of multidrug resistance bacteria were found in patients agedabove 50 years and were prevalent in OPD, emergency and gynae wards. The dominant bacterial species were gram negative, *Escherichia coli* (29%), *Staphylococcus aureus* (19%), *Pseudomonas aeruginosa* (13.33%), *Acinetobacter* species (5.71%), whereas, gram negative isolates were *Staphylococcus epidermidis* (9.52%), *Streptococcus* specie (5.71%), and *Enterococcus faecium*. Antibiotics like amoxicillin/ clavulanic acid, cefuroxime and sulphamatoxazole/ trimethoprim were resistant to 64.61%, 63.07% and 61.53% of gram-negative bacteria respectively while ciprofloxacin, doxycycline and fusidic acid were resistant to 70%, 52.5% and 52.5% gram positive bacteria respectively. The most susceptible antibiotics against gram negative were sulbactum/ cefoperazone and amikacin while to gram positive were linezolid, chloramphenicol and rifampicin **Conclusion:** Current study revealed increasing antibiotic resistance pattern that need intimidate focus on surveillance of antibiotics resistance regularly and to ensure long lasting efficacy of antibiotics.

INTRODUCTION

Efforts intended at identifying new antibiotics were once a top research and development primacy among pharmaceutical industries. The powerful broad-spectrum drugs that appeared from these accomplishments provided extraordinary clinical efficiency but success has been compromised. Now we are facing a long list of microorganisms that have shown resistance for many classes of drugs and are no longer susceptible to most, if not all, antibiotics [1]. The development and antibiotics usage have been one of the key scientific triumphs of the 20th century. The bacterial infections were considered to be under control during the early period of antibiotic usage [2]. At that time there were no alarm of cuts and infection, and various bacterial diseases, such as cholera and syphilis were considered on their way to eradication [3]. However, extensive antibiotics usage has upheld the antibiotic-resistant pathogens. Resistance spreading promptly, predominantly in hospitals, where different bacteria may come in close contact with each other and providing the environment for distributing the resistant genes with other bacteria [4,5]. Bacterial infections due to both gram positive and negative bacteria have caused huge causalities. Most gram negative pathogens such as Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter spp. make hindrance in treatment by developing different strategies [6]. The use of antibiotics in any environment produces selection forces that favor the survival of antibiotic resistant pathogens [7].

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Vatopoulos and Kalapothaki, (1999) [8] reported that Escherichia coli, K. pneumoniae, Enterobacter spp, P. aeruginosa, Acinetobacter baumanii and Staphylococcus aureus have shown resistivity patterns to different pathogens. And these pathogens have been isolated from nosocomial or outpatient in most parts of the world because of their rate of isolation, pathogenicity and virulence [8]. It is obvious that we are at a critical time in the history of medicines, where the genetic variations and acquirements from environmental sources by bacteria may leave us with no lifesaving therapeutics options [9]. Keeping in view the emergence of resistance in bacterial pathogens and due to limited options of treatment, present study was designed to find the prevalence of antibiotics resistant bacteria in tertiary care hospital sittings and to design strategies to control the spread of resistant bacteria.

METHODS

Collection of samples and specimens: In this study total 753 specimen samples were collected from patients who were admitted or visited tertiary care hospital Peshawar. Clinical history, informative details and demographic features like age and gender of each patient were noted. Different clinical culture and sensitivity test samples were taken from urine, wound (pus) swab, sputum samples, blood etc. and were transported on ice bag and glycerol stock to the Department of Microbiology, Kohat University of science and technology for further analysis. This research was conducted from May, 2017 to October, 2017.

Bacteriological analysis: The samples were streaked on culture media for isolation and growth. Then all the samples except urine samples were streaked on MacConkey to differentiate between gram positive and gram negative bacteria and also to examine lactose and non-lactose fermenting bacteria. Urine samples were streaked on Cystine Lactose Electrolyte-deficient (CLED) medium. Blood agar plates were used to differentiate fastidious bacteria especially *streptococcus* species. Hemolysis were observed, alpha hemolysis showed, *Streptococcus aureus* while beta and gamma hemolysis indicated *Streptococcus pneumoniae*. All the cultures were incubated for 24hours [8]. For morphological identification the isolates were grown on agar media and their growth patterns, shape and color were observed. Gram staining was performed to further differentiate bacteria based on staining and shape.

Biochemical tests: For biochemical characterization tests like catalase, triple sugar ion (TSI), motility, indole, urease and oxidase were performed and according to Bergey's Manual of systematic Bacteriology, on the basis of morphological, physiological and biochemical features, characterization was done [10].

Antibiotic susceptibility patterns: By using Kirby-Bauer disc diffusion method [11], following the recommendation of the clinical and laboratory standard institute[12] the antibiotic resistivity of bacterial isolates was assisted. With addition of 5% sheep blood, Mullen-Hinton agar was prepared. A suspension of bacteria was made and then streaked on agar plate. Antibiotic disks were applied and then incubated at 37°C for 24 to 48 hours. The following antibiotics were used for gram positive: cefoxitin (FOX), erythromycin (E), clindamycin (CL), gentamycin (CN), vancomycin (VA), linezolid (LZD), chloramphenicol(C), teicoplanin (TEC), amoxicillin/clavulanic acid (AMC), penicillin (PEN), ciprofloxacin (CIP), rifampicin (RD), fusidic acid (FD), doxycycline (DO), polymyxin-B (PB), andsulphamatoxazole/trimethoprim (SXT). For gram negative bacteria cefotaxime (CT), piperacillin/tazobacterum (TZB), ceftazidime (CAZ), CN, sulbactum/cefoperazone (SCF), colistin sulfate (CST), meropenem (MEM), Imipenem (IPM), minocycline (MIN), amikacin (AK), tigecycline (TGC), ceftriaxone (CRO), cefuroxime (CE), nitrofurantoin and fosfomycin were used. All these antibiotic disks were purchased from Oxoid, UK. After 48 hours of incubation, the diameters of zone of inhibition around the antibiotic disks were measured by using graduated ruler and interpreted the results according to CLSI guidelines [20].

RESULTS

Isolation and distribution of bacteria: The specimen wise distribution of the isolates was 29.5% (n= 31) from pus, 38% (n=40) from urine and 32.3% (n=34) from swab samples. Gender wise distribution of the isolates were 67.6% (n=71) from male and 32.4% (n=34) from female while age wise distribution was 8.5% (n=9), 13.3% (n=14), 47.6% (n=50) and 30.4% (n=32) from <10 years, teenagers, adults and senior citizens respectively as shown in Table 1. While Table 2 shows the frequency of patients that were admitted at different wards like OPD (n=42), ENT B (n=3), emergency (n=38), PEADS (n=1), gynae A (n=8), gynae C (n=2), gynae OPD (n=3), ENT OPD (n=1) and endocrinology (n=2) at Hayatabad Medical Complex Peshawar.

Specimen wise distribution	
Pus	29.5%
Urine	38%
Swab	32.3%
Gender wise distribution	
Male	67.6%
Female	32.4%
Age wise distribution	
<10 years	8.5%
Teenagers	13.3%
Adults	47.6%
Senior citizens	30.4%

Table 1: Distribution of the bacterial isolates based on specimen,

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gender and age.

Essential oil and standard drug were tested against Staphylococcus aureus *(S. aureus)*, Bacillus subtilis *(B. subtilis)*, Pseudomonas aeruginosa *(P.aeruginosa)* and Escherichia coli *(E. coli)*. Cumin essential oil showed significant antibacterial activity against both gram-positive and gram-negative bacterial strains. Standard drug data showed that it was effective against *S .aureus* and least against *E.coli* which is a resistant gram negative strain.

Ward	Frequency
OPD	42
ENT B	3
Emergency	38
PEADS	1
Gynae A	8
Gynae C	2
Gynae OPD	3
ENT OPD	1
Endocrinology	2

Table 2: Wards wise distribution of patients' clinical specimens

Isolates	Biochemical Tests								
	G. staining	Shape	Catalase	Coagulase	Oxidase	TSI	M	I	U
E.coli	-	Rods	+	-	-		+	+	-
P. aeruginosa	-	Rod	+	-	+	-	+	-	-
S. aureus	+	Cocci	+	+	-	+	-		+
S. epidermidis		Cocci	+	-	-		-		+
Streptococcus species	+	Cocci	-				-		-
E. faecium	+	Cocci	-	-	-		-	-	-
Enterobacter species	-	Rod	+		-		+	-	-
P. mirabilis	-	Rod	+		-		+	-	+
Acinetobacter	-	bacillus	+		-		-	-	-
Providencia . species	-	Straight rods	+		-		+	+	+

Table 3: Biochemical and Morphological characteristics of resistant bacterial isolates TSI, triple sugar iron, M, Motility, I, Indole, U, Urease

Among the total of 105 bacterial isolates, 61.90% (n=65) were gram negative and38.1%(n=40) were gram positive. The biochemical testsare given in Table 3 used for the identification process. *E. coli* was the most frequently found gram negative bacteria whereas *S. aureus* was most frequently found gram positive bacteria. Overall frequency of *E.coli*(29%) was high, followed by *S.aureus*(19.4%), and *P. aeruginosa*(13.33%). Among less frequently found bacteria, the Acinetobacter specie (5.71%), Enterobacter, Coliforn and Proteus mirabellis (3.80%) and Providencia species (2.85%) were gram negative whereas, *S.epidermidis* (9.52%), Streptococcus species(5.71%), and Enterococcus faecium(3.80%)were gram positive as shown in Table 4.

Isolates	No. of Isolates	No. of Isolates %
E.coli	30	29%
S. aureus	20	19.04%
P. aeruginosa	14	13.33%
S. epidermidis	10	9.52%
Actineobacter species	6	5.71%
Streptococcus species	6	5.71%
Enterobacter species	4	3.80%
Coliforn species	4	3.80%
P.mirabellis	4	3.80%
Enterococcusfaecium	4	3.80%
Providencia species	3	2.85%

Table 4: Prevalence of Gram positive and Gram negative Resistant Bacterial isolates

Antibiotics resistance profile of Bacterial Isolates: Antibiotic susceptibility of gram negative and gram positive bacteria are shown in Figure 1 and Figure 2 respectively. The AK (73.84%) followed by SCF (70.6%) are most sensitive and then TZB (69.2%) and MEM (67.6%) were found effective antibiotics against Gram negative isolates shown in Table 5 and LZD (87.5%), C (85%) and RD (82.5%) were found effective antibiotics against gram positive isolates as shown in Table 4. The most resistant antibiotics against gram negative isolates were CTX (58.46%), CAZ (56.92%), SXT (61.53%), CE (63.07%), and AMC (64.61%) while against gram positive were SXT (47.5%), E (50%), DO (52.5%), CIP (70%) and FD (52.5%) as shown in Table 5 and 6 respectively.

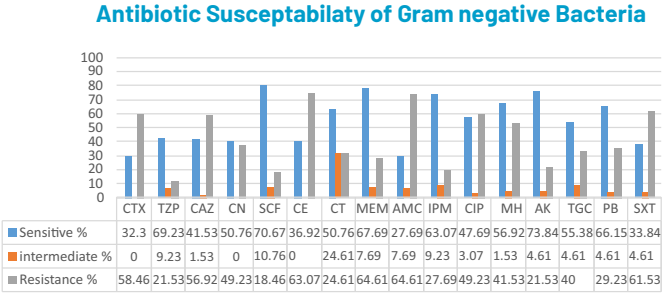

Figure 1: Antibiotic susceptibility of gram negative isolates

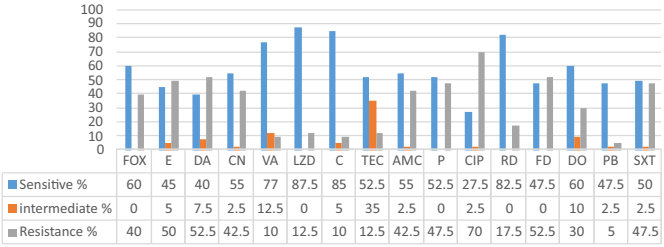

Figure 2: Antibiotic susceptibility of gram positive isolates

Antibiotics discs	Sensitive %	intermediate%	Resistance%
CTX	32.3	0	58.46
TZP	69.23	9.23	21.53
CAZ	41.53	1.53	56.92
CN	50.76	0	49.23
SCF	70.67	10.76	18.46
CE	36.92	0	63.07
CT	50.76	24.61	24.61
MEM	67.69	7.69	24.61
AMC	27.69	7.69	64.61
IPM	63.07	9.23	27.69

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CIP	47.69	3.07	49.23
MH	56.92	1.53	41.53
AK	73.84	4.61	21.53
TGC	55.38	4.61	40
PB	66.15	4.61	29.23
SXT	33.84	4.61	61.53

Table 5: Prevalence of gram negative antibiotic susceptible isolates

Antibiotics	Sensitive %	Intermediate %	Resistance %
FOX	60	0	40
E	45	5	50
DO	40	7.5	52.5
CN	55	2.5	42.5
VA	77	12.5	10
LZD	87.5	0	12.5
C	85	5	10
TEC	52.5	35	12.5
AMC	55	2.5	42.5
P	52.5	0	47.5
CIP	27.5	2.5	70
RD	82.5	0	17.5
FD	47.5	0	52.5
DA	60	10	30
PB	47.5	2.5	5
SXT	50	2.5	47.5

Table 6: Prevalence of gram positive antibiotic susceptible isolates

DISCUSSION

Infectious diseases are the result of host invasion with a pathogen. The detection, treatment and prevention of human diseases are the challenges physicians, pharmacists and microbiologists are facing. In our total studied clinical samples 38% urine, 32% wound and 30% swabs samples were collected from patients. Similar specimen samples were also studied by Khurshid et al., (2002) [13] at Ayub Medical College, Pakistan. Gram negative pathogens were more prevalent in our study which is correspondence with a study conducted at tertiary general hospital China where 59% gram negative isolates were observed [14]. In our study the majority of infections are due to E. coli29%followed byS.aureus19.04%. In another study it is reported that S.aureusas 18.5% and E. coli as 16.7% isolated from nosocomial infection patients [15]. The most susceptible antibiotic against gram negative bacteria were AK and SCF while against gram positive were LZD, C and RIF. Similar studies on antibiotic susceptibility profiling were conducted by Sekhar, et al (2014) [16] in India where all gram positive isolates were sensitive to DO while gram negative to AK, SCF, and MEM and George et al (2018) [17] who reported IMP as most effective antibiotic against gram negative while VA and CL were effective against gram positive. Antibiotics are notorious to put selective pressure on antibiotic susceptible bacteria and boost the development of antibiotics resistant. It is therefore a possible reason of little disagreement with other studies [18]. Moreover, high antibiotic resistance rates were observed in gram negative bacteria against CE, AMC and SXT while in gram positive against CIP and DO. In a previous pilot study resistance against AMC was observed in gram negative isolates while against PEN to gram positive isolates [19]. The changes in bacterial resistance profile are may be due to the close relationship of bacteria with each other and their easy acquirement of genes in different environments [20].

CONCLUSIONS

It is concluded that the most prevalent pathogens in our study were E.coli (29%), S.aureus (19.4%) and P. aeruginosa (13.33%) and higher incidence were observed in urine specimens, male and old age people. The AK and SCF in gram negative while LZD, Cand RIF were found most susceptible while CE, AMC and SXT in gram negative and CIP and DO in gram positive were found most resistant antibiotics. So knowledge of the clinical, bacteriological finding as well as antibiotic susceptibility profiles are essential for choice of appropriate antibiotic with maximum effectiveness in correct management of the patient and to reduce the risk of complications.

REFERENCES

[1] Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell. 2007;128(6):103750.doi.org/10.1016/j.cell.2007.03.004

[2] French GL. The continuing crisis in antibiotic resistance. Int J Antimicro Agents. 2010;1;36:S3-7.doi.org/10.1016/S0924-8579(10)70003-0

[3] Bodimeade C, Marks M, Mabey D. Neglected tropical diseases: elimination and eradication. Clin Med. 2019;19(2):157.doi: 10.7861/clinmedicine.19-2-157

[4] Juan CH, Chuang C, Chen CH, Li L, Lin YT. Clinical characteristics, antimicrobial resistance and capsular types of community-acquired, healthcare-associated, and nosocomial Klebsiella pneumoniae bacteremia. Antimicrob Res Infect Control. 2019;8(1):1-9.doi.org/10.1186/s13756-018-0426-x

[5] Phodha T, Riewpaiboon A, Malathum K, Coyte PC. Excess annual economic burdens from nosocomial infections caused by multi-drug resistant bacteria in Thailand. Ex Rev Pharmacoec Outcomes Res. 2019;19(3):30512.doi.org/10.1080/14737167.2019.1537123

[6] D'Costa VM, McGrann KM, Hughes DW, Wright GD. Sampling the antibiotic resistome. Science.

DOI: https://doi.org/10.54393/pbmj.v5i3.177

2006;311(5759):374-7. doi: 10.1126/science.1120800
[7] Klemm EJ, Wong VK, Dougan G. Emergence of dominant multidrug-resistant bacterial clades: Lessons from history and whole-genome sequencing. Proc Natl Acad Sci. 2018;115(51):12872-7.doi.org/10.1073/pnas.1717162115
[8] Vatopoulos AC, Kalapothaki V, Legakis NJ. An electronic network for the surveillance of antimicrobial resistance in bacterial nosocomial isolates in Greece. The Greek Network for the Surveillance of Antimicrobial Resistance. Bull World Health Org. 1999;77(7):595.
[9] Giamarellou H. Treatment options for multidrug-resistant bacteria. Exp Rev Anti-infective Therapy. 2006;4(4):601-18.doi.org/10.1586/14787210.4.4.601
[10] Parte A, Whitman WB, Goodfellow M, Kämpfer P, Busse HJ, et al. editors. Bergey's manual of systematic bacteriology: volume 5: the Actinobacteria. Springer Science & Business Media; 2012 Jun 23.
[11] Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol 2009.
[12] Hsueh PR, Ko WC, Wu JJ, Lu JJ, Wang FD, Wu HY, et al. Consensus statement on the adherence to Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing Guidelines (CLSI-2010 and CLSI-2010-update) for Enterobacteriaceae in clinical microbiology laboratories in Taiwan. J Microbiol, Immunol Infect. 2010;43(5):452-5. doi: 10.1016/S1684-1182(10)60070-9
[13] Khurshid R, Sheikh MA, Karim S, Munnawar F, Wyne H. Sensitivity and resistance of antibiotics in common infection of male and female. J Ayub Med Coll Abbottabad. 2002;14(1).
[14] Wang M, Wei H, Zhao Y, Shang L, Di L, et al. Analysis of multidrug-resistant bacteria in 3223 patients with hospital-acquired infections (HAI) from a tertiary general hospital in China. Bosnian J Basic Med Sci. 2019;19(1):86. doi: 10.17305/bjbms.2018.3826
[15] Tolera M, Abate D, Dheresa M, Marami D. Bacterial nosocomial infections and antimicrobial susceptibility pattern among patients admitted at HiwotFana Specialized University Hospital, Eastern Ethiopia.AdvMed.2018;4;2018.doi.org/10.1155/2018/2127814
[16] Sekhar SM, Vyas N, Unnikrishnan MK, Rodrigues GS, Mukhopadhyay C. Antimicrobial susceptibility pattern in diabetic foot ulcer: a pilot study. Ann Med Health Sci Res. 2014;4(5):742-5.dx.doi.org/10.4103/2141-9248.141541
[17] George M, Iramiot JS, Muhindo R, Olupot-Olupot P, Nanteza A. Bacterial aetiology and antibiotic susceptibility profile of post-operative sepsis among surgical patients in a tertiary hospital in rural Eastern Uganda. Microbiol Res J Inter. 2018;24(2). doi: 10.9734/MRJI/2018/41690
[18] Taha AB. Relationship and susceptibility profile of Staphylococcus aureus infection diabetic foot ulcers with Staphylococcus aureus nasal carriage. The Foot.2013;23(1):116.doi.org/10.1016/j.foot.2012.10.003
[19] Li X, Ding X, Shi P, Zhu Y, Huang Y, Li Q, et al. Clinical features and antimicrobial susceptibility profiles of culture-proven neonatal sepsis in a tertiary children's hospital, 2013 to 2017. Medicine. 2019;98(12).doi: 10.1097/MD.0000000000014686
[20] Hombach M, Bloemberg GV, Böttger EC. Effects of clinical breakpoint changes in CLSI guidelines 2010/2011 and EUCAST guidelines 2011 on antibiotic susceptibility test reporting of Gram-negative bacilli. J Antimic Chemoth. 2012;67(3):622-32. doi.org/10.1093/jac/dkr524