

BACTERIAL PROFILE AND ANTIBIOGRAM OF MICROORGANISMS ISOLATED FROM DIFFERENT BODY SITE INFECTIONS AMONG PATIENTS OF A PRIVATE HOSPITAL, KARACHI

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ABSTRACT

Objective: It is crucial to keep an eye on current patterns of multi-drug resistance and extensive drug resistance at local level to facilitate physicians in making justified decisions regarding empirical therapy during this era of rising superbugs.

Material and Methods: This antibiogram was made up according to CLSI (Clinical Laboratory Standard Institute) M100 guidelines. Samples were received for a period of 2-years from June 2019-June 2021 at National Medical Center Karachi. These samples were urine, blood, pus, cerebrospinal fluid (CSF), central venous pressure (CVP) tip, sputum and tracheal aspirates.

Results: Out of 10564 samples received, 4582 were positive for growth of microorganisms. Sample distribution patterns according to the frequency of positive growth of isolates were predominantly urine (58%) and blood (17%) followed by pus (8%) and pus swab (6%), fluid and tracheal secretions (2%), and others like tips growth (7%). Blood cultures were mainly received from intensive care unit (50%) followed by out-patients departments (34%). The pattern of resistance observed in *Escherichia coli* (*E. coli*) revealed ampicillin as the most resistant among all antibiotics (93%) followed by cefixime (77%). Most sensitive antibiotic was amikacin (99%). The *Staphylococcus aureus* isolates were highly susceptible to vancomycin and linezolid (100%) followed by gentamicin (89%) and chloramphenicol (85%). Vancomycin resistance was observed in *Enterococcus faecium* (8%).

Conclusion: Antimicrobial resistance among gram negative and gram-positive bacteria is increasing. This has left us with fewer therapeutic options which in turn highlights the issue of antimicrobial resistance being a global health concern. This emphasizes the significance of surveillance of antimicrobial patterns, development of antibiograms and implementation of antibiotic stewardship programs by judicious use of antibiotics for prevention of further spread of antimicrobial resistance.

Key Words: Antibiogram, Antimicrobial resistance, Multidrug resistance, Extensively drug resistance

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INTRODUCTION

Bacterial infections remain the major cause of morbidity and mortality worldwide [1,2]. Antimicrobial resistance (AMR) imposes a significant threat to public health all over the world. It has a drastic, negative impact on people's lives. The threat of antibiotic resistance has emerged as a global health crisis that could result in 10 million deaths every year by 2050. Currently, due to AMR at least 50 000 lives per year are lost in Europe and the United States of America. The growing incidence of antimicrobial resistance may be attributed to irrational use of antibiotics, inappropriate dosage and the absence of standardized protocols for antibiotic use [1-3].

Inadequate resources, poor microbiology services, lack of technical expertise in local laboratories and insufficient epidemiological data, all contribute to the increase in burden of severe bacterial infections in developing countries like Pakistan [4]. The World Health Organization (WHO) in 2015 established The Global Action Plan on Antimicrobial Resistance which was endorsed by most countries including Pakistan. This includes imparting knowledge of antimicrobial resistance, surveillance, reducing the incidence of infections through infection control measures; and investment in newer antibiotics, diagnostic modalities, and other medical interventions [5]. Pakistan is the first country to take an initiative towards early implementation of antimicrobial resistance surveillance system in the *Eastern Mediterranean Region* [6,7]. It is imperative to develop awareness regarding antimicrobial stewardship, current therapeutic options and last resort antimicrobials in

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intensive care settings for effective empirical treatment [6-8].

This study is a methodical and systematic approach to present the antibiotic susceptibility and resistance pattern of different nosocomial isolates as well as isolates from community acquired infections. The objective of this study was to find out the types and frequency of microorganisms which exhibit resistance to determine the antibiotic susceptibility profile so as to help in implementation an empirical therapy protocol.

MATERIAL AND METHODS

This retrospective cross-sectional study was done at microbiology laboratory of National Medical Center, Karachi. Sample size was estimated by Gpower version 3.1.9.2 software at 95% confidence level and 5% margin of error. Calculated sample size was 4582. Out of total 10564 samples received, 4582 were positive for growth of microorganisms. One investigator extracted the data from June 2019 to June 2021 through the lab LIMS system (Laboratory information Management System) with permission of hospital management using non-probability consecutive sampling technique. During this time period 10564 samples were received and 4582 were positive for bacterial growth. Only those microorganisms (*Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus (faecalis) (state species)*) were included in this study which were found more frequent and increase the burden for selection of antimicrobials. Those positive growths presented with contamination and mixed growth were excluded from study. Samples were covered from urine, blood, pus, pus swab, and fluid, tracheal secretions and others like catheter tips. Phenotypic antimicrobial susceptibility patterns were seen on isolates from clinical samples of adult and pediatric patients hospitalized in the medical, surgery, and Intensive Care (ICU) units. Samples were also received from outpatient departments. Clinical specimens included in the study consist of urine, blood, pus, pus swab, central venous pressure (CVP)tip, sputum and tracheal aspirates. Blood samples were processed in BACTEC automated blood culture system and once they were positive, gram staining was done and samples were sub-cultured on blood, Mac-Conkey, chocolate agar. Other specimens were processed as per standard microbiological protocols. Isolate identification was carried out by performing biochemical tests based on interpretation of gram stain. Final identification was done by API 20E and

API 20NE (Biomerieux) and confirmed by MicroScan (Beckman coulter) ®. The antimicrobial susceptibility was performed by standard susceptibility test using the Bauer–Kirby disk diffusion method. Antimicrobial disc contents were as follows: amikacin 30µg (oxid), gentamycin 10µg (oxid), ceftriaxone 30µg (oxid), ceftazidime 30µg (oxid), cefepime 30µg (oxid), piperacillin/ tazobactam 100/10µg (oxid), imipenem 10µg (oxid), meropenem 10µg (oxid), ciprofloxacin 5µg (oxid), vancomycin 30µg (oxid). Cefoxitin (30-µg) disc was used for detection of Methicillin resistant *Staphylococcus aureus* (MRSA) according to data record. Minimum inhibitory concentration (MICs) of Polymyxin B was done by broth microdilution using sensititre-plates (Thermo-scientific™). Vancomycin susceptibility for *Staphylococcus aureus* was done by agar dilution method. Antibiotic susceptibility interpretation was based on the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing M100 criteria [9]. Control strains used were *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 (Liofilchem, Italy). The data collected was analyzed using descriptive statistics performed with Statistical Package for the social sciences (SPSS) version 24:00. Mean +/- SD was calculated for continuous variables. Frequency and percentage was calculated for categorical variables. Chi-square was used to test for statistical significance ($p \leq 0.05$).

RESULTS

Out of 10564 samples received 4582 samples were found positive for growth of different microorganisms. Distribution pattern of various specimens yielding positive growth of microorganisms was urine (58%), blood (17%) followed by pus (8%) pus swab (6%) and 2% fluid and 2% tracheal secretions and 7% others like tips growth as mentioned in Figure-1. Positive blood cultures received were mainly from Intensive care unit (50%), out patients' department (34%) followed by other in door-patient (16%). Distribution of different microorganisms from various specimens was as follows: *Escherichia coli* from urine (75%), *Salmonella enteric* serovar Typhi (S.Typhi) from blood culture (52%), *Staphylococcus aureus* from pus culture (56%), *Acinetobacter baumannii* from blood (48%), *Klebsiella pneumoniae* from tracheal aspirates (46%) and *Enterococcus* from blood (42%). Overall frequency of microorganisms from various specimens was as follows: *Escherichia coli* 2231(49%), *Salmonella enteric* serovar Typhi (S. Typhi) 412 (9%), *Staphylococcus aureus* 379 (8%),

Acinetobacter baumannii 340 (7%), *Klebsiella pneumonia* 732 (16%) and *Enterococcus* 92(2%), *Pseudomonas aeruginosa* 396 (9%). The pattern of resistance observed in *E.coli* revealed ampicillin as the most resistant (98%) among all antibiotics followed by cefixime (77%) and ceftriaxone (70%). Most susceptible antibiotic was amikacin (99%). The *Staphylococcus aureus* (*S. aureus*) isolates were highly susceptible to vancomycin and linezolid (100%) followed by gentamicin (89%) and

chloramphenicol (85%). Frequency of Methicillin resistant *Staphylococcus aureus* (MRSA) was 67%. Vancomycin resistance was observed in *Enterococcus faecium* (8%). Table-I indicates the susceptibility pattern of different microorganisms to various antimicrobials.

Table-I: Susceptibility pattern of different microorganisms to various antimicrobials (n=4582).

	Percentage (%)	<i>E. coli</i>	<i>Acinetobacter baumannii</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>S. Typhi</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus spp</i>	
AK	Sensitive	99	28	47	83	-	-	-	0.000
Amikacin									
AMC	Sensitive								
Amoxicillin-clavulanate		34		20			33		0.026
AMP	Sensitive	7							
Ampicillin						28	11	45	0.000
TZP	Sensitive								
Piperacillin-tazobactam		65	15	32	86				0.000
CFM	Sensitive								
Cefixime		23	1	26					0.000
CIP	Sensitive								
Ciprofloxacin		27	4	26	85	13	33		0.000
CN	Sensitive								
Gentamicin		72	22	35	83		89		0.000
CRO Ceftriaxone	Sensitive	30				71			0.004
FOS	Sensitive	67							0.000
Fosfomycin									
IPM	Sensitive	70	25	68	89				0.000
Imipenem									
MEM	Sensitive	71	38	35	90	100			0.000
Meropenem									
PB	Intermediate	99		98	100				0.364
Polymyxin B									
CAZ	Sensitive	20			85				0.000
Ceftazidime									
SXT	Sensitive					66			
Trimethoprim-sulfamethoxazole				34			64		0.000
ATM	Sensitive								
Aztreonam				18	78				0.000
C	Sensitive					48			
Chloramphenicol							85		0.001
P	Sensitive						11	45	0.000
Penicillin									
VA	Sensitive						100	92	0.004
Vancomycin									

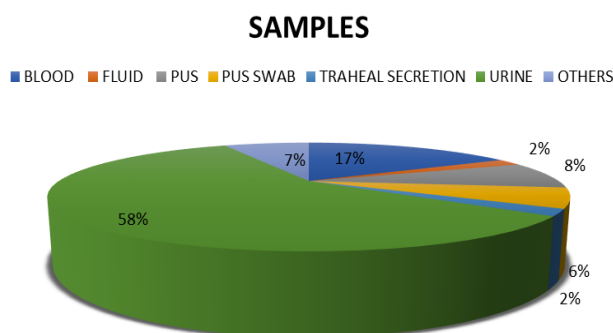


Figure-I: Distribution of various clinical specimens (n= 4582).

DISCUSSION

Antibiotic resistance of pathogens is a global issue, because it has a detrimental impact on people all over the world, especially developing countries [10]. In April 2018, the Pakistan Global Antibiotic Resistance Partnership published a situation analysis report about antimicrobial resistance in Pakistan [11] which concluded that major challenges include a multitude of unnecessary registered products, misleading advertisements, increasing number of quacks, irrational drug prescribed by clinicians, availability of over-the counter drugs without prescription, bias attitude of physicians towards costly broad-spectrum antibiotics and widespread

use of antibiotics in animals and agriculture industry [11]. To address the issue of antimicrobial resistance, a multifactorial approach along with involvement of administration is required. Knowing the frequency of microorganisms isolated from different clinical specimens and their associated infections is mandatory for treatment of infectious diseases and monitoring of antimicrobial resistance. The current study aimed at establishing the frequency of common pathogens and their antimicrobial susceptibility patterns, their distribution according to specimens, at National Medical Centre, Karachi.

In our study Gram-negative bacteria were the predominant bacteria among all clinical specimens. This finding is consistent with reports from all over the world, where gram-negative bacteria were more frequently found [12]. Gram positive pathogen *S.aureus* is one of the common causes of skin and soft tissue infections [11]. In our study we observed considerably high number of *S.aureus* isolates from pus and pus swab specimens, obtained from wound and surgical site infections. The prevalence of ceftriaxone (70%) and cefixime (77%) resistance was remarkable in *E.coli* isolated from different samples in our study. This observation may be due to non-judicial and inappropriate use of ceftriaxone. Ceftriaxone being a safer drug option makes it more vulnerable towards its misuse contributing to development of resistance [14]. *Enterococcus spp* exhibited 55% resistance against ampicillin and penicillin. This is in accordance with the study conducted in China in 2020 [15] where 40% isolates showed resistance against penicillin. Our data exhibited 100% sensitivity against linezolid and 92% susceptibility against vancomycin. These results show a slight contrast in susceptibility in vancomycin and linezolid in study carried out in China, this may be a red flag for clinician's specially managing critically ill patients [16]. Oral availability of linezolid is one of the factors observed contributing to its resistance observed in study conducted in France [17] although our data exhibits 100 % sensitivity against linezolid. Carbapenem resistance is one of the striking observations in our study, which is so alarming in the era of developing antibiotic resistance. Resistance to carbapenems in *E.coli* was 43% and in *Acinetobacter baumannii* it was found up to 80%. There is a remarkable correlation between antibiotic consumption and developing resistance in previous few years with the study conducted in China [18]. Our results show strong positive association with the multicenter study conducted in China exhibiting carbapenem resistance in *Enterobacteriaceae* and *A. baumannii* [19]. CRAB (Carbapenem resistant *Acinetobacter baumannii*) is

on the verge of rising due to non-judicial use of carbapenems in in-patients and intensive care units [20, 21]. Carbapenem is the only treatment option for extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae*. The rate of resistance against cephalosporins has been increasing in *Enterobacteriaceae* due to ESBL production in a study observed in Korea [22]. Enteric fever is endemic in Pakistan and Southeast Asia [23]. Major challenges faced in treatment of enteric fever is spreading MDR (multi-drug resistant) and XDR (extensively drug resistant) strains of *Salmonella enteric* serovar Typhi. Major outbreak of XDR salmonella has been observed in 2018 in Hyderabad [24]. In our study we have observed a striking increase in Ceftriaxone resistant XDR-salmonella isolates (29%) which is comparable to study conducted in Kenya [25]. Only treatment options left for such isolates are meropenem and azithromycin. Colistin has re-emerged as a last resort after 1990s against MDR Gram-negative microorganisms due to the development of extensively drug-resistant superbugs. Unfortunately, global resistance towards colistin has increased following its misuse [26]. Being a multidrug-resistant microorganism, *Acinetobacter baumannii* (*A. baumannii*) is one of the major causes of hospital acquired infections in the current healthcare system. Increasing evidence of extensively drug-resistant (XDR) isolates of *A. baumannii* is also observed in different countries [27]. The World Health Organization (WHO) has attributed *A. baumannii* as a critical priority pathogen has been imposing a huge threat to human health. Newer antibiotic options effective against *A. baumannii* are urgently required [28]. All antimicrobials exhibited more than 80% resistance against *A. baumannii* which is an eye opener for the clinicians. These results are in accordance with the study conducted in Belgium [29]. Minimum inhibitory concentrations of polymyxin B done by broth microdilution revealed that 80 percent isolates have MIC <0.5 μ g/ml followed by 8% having <1 μ g/ml. These results are in accordance with an international study. However slight difference observed is due to change in laboratory detection method and demographic data [30].

CONCLUSION

Growing antimicrobial resistance among gram negative and gram-positive isolates with fewer therapeutic options in various microorganisms was observed in our study which highlights the idea that antimicrobial resistance is a global health concern. This reinforces the significance of surveillance of antimicrobial patterns, development of antibiograms

and implementation of antibiotic stewardship programs by judicious use of antibiotics for prevention of further spread of antimicrobial resistance.

AUTHOR CONTRIBUTION

Shaista Bakhat, Saman Nadeem: Study design, literature search, data collection, data interpretation, writeup

Yasmeen Taj: Study design, data collection, data interpretation

Beenish Hussain: Literature search, data collection, data analysis, proofreading

Ashfaq Hussain: Study design, data collection, data interpretation, proofreading

Danish Shakeel: Literature search, data analysis and interpretation, writeup

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