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Research Article

Susceptibility Pattern of Isolates from Surgical Ward Patients of A Tertiary Care Referral Hospital, Rawalpindi, Pakistan

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Abstract

Introduction: Surgical site infections are prevalent in hospitals all across the country. Lack of antibiotic stewardship and injudicious use has led to emergence of multidrug resistant organisms which makes treatment of hospital acquired infections a daunting challenge for the physicians. The trend of isolates and their sensitivity pattern remains in a constant state of flux therefore regular monitoring is mandatory for formulating effective antibiotic policies.

Objective: The aim of the study was to find out the susceptibility pattern of isolates from surgical ward patients of a tertiary care referral hospital in Rawalpindi, Pakistan.

Materials and methods: This descriptive study was conducted at surgical units of Military Hospitals Rawalpindi from January through December 2014. Samples received from infected wounds of admitted patients were included in the study. The isolates were identified by biochemical reactions and antibiotic sensitivity testing was performed by Modified Kirby-Bauer disc diffusion technique according to CLSI zone interpretive criteria.

Results: Among Gram positive organisms, *Staphylococcus aureus* was significant isolated from 185 (24.15%) cases. Among these isolates 97(12.7%) were methicillin resistant. Total number of Gram negative organisms isolated were 472 (61.6%) and Gram positive constituted 294 (38.4%). The most frequent Gram negative organisms isolate was *Escherichia coli* 162(21.14%) followed by *Pseudomonas aeruginosa* 115(15%), *Klebsiella pneumonia* 56(7.31%) and *Acinetobacter baumannii* 54(7.15%).

Conclusion: In our setup Gram negative organisms were the predominant group involved in infections of patients admitted in surgical units.

Keywords: *Staphylococcus aureus; Klebsiella pneumonia; Acinetobacter baumannii; Escherichia coli*

Introduction

Inappropriate and injudicious use of broad spectrum antibiotics has led to the emergence of multidrug-resistant (MDR) bacteria [1]. The resistance in bacteria against antibiotics is either intrinsic or acquired by transfer of mobile genetic elements among bacteria sharing a common habitat [2]. In a country like ours where hospital infection control measures are still rudimentary and no antibiotic restriction, the situation is even more alarming.

Surgical site infections (SSIs) are the third most common form of hospital acquired infections [3]. These infections affect 2-5% of patients undergoing surgical procedures in the United States each year with resultant 500,000 infections; 3.7 million more days stayed in the hospital and costing$1.6 billion. Hospital resident flora is exposed to all kinds of antibacterial agents resulting in higher level of resistance as compared to strains of the same bacteria in the community. The pattern and diversity of bacterial isolates vary in different geographical regions of the world. Infections caused by MDR Gram negative bacilli (GNB) are becoming more common as compared to Gram positive organisms in some setups while Gram positive pathogens still constitute majority of isolates in surveillance studies conducted at other establishments [3,4].

Highly resistant Gram positive and GNB are a serious health concern in surgical wards of tertiary care military hospital, Rawalpindi. Published data on the spectrum and susceptibility profile of bacterial isolates is sparse and needs regular updating. The dearth of published data leads to irrational use of antibiotics which puts unnecessary strain on financial resources and also leads to increased emergence of resistance in resident flora through exchange of resistance plasmids and other mechanisms. This study was conducted to ascertain the current sensitivity profile of the various organisms isolated from patients admitted to surgical wards of military hospitals at Rawalpindi.

Materials and Methods

The study was conducted at Department of Microbiology, Armed Forces Institute of Pathology, and Rawalpindi, Pakistan. All pus and pus swab samples from various surgical units of local tertiary care military hospitals sent for bacterial culture from January through December 2014 were included in the study. Repeat samples from same patient and those contaminated during collection and transport were not included in the study. The surgical ward staff was directed about correct procedure for collection and transport of pus specimen.

All samples were inoculated on 5% sheep blood agar (Oxoid, UK), MacConkey agar (Oxoid, UK) and Wilkins Chalgren agar (Oxoid, UK) and incubated at 37°C aerobically and anaerobically according to the media. Gram and Ziehl-Neelsen staining of the sample slides was done in each case. The plates were examined after 24 hours and again after 48 hours for any visible growth. The organisms were identified by colony characteristics, Gram stain and rapid tests (catalase, oxidase, DNAse and coagulase). Biochemical identification of GNR was done through API20E/NE (Biomerieux, France). Antibiotic sensitivity testing was done by modified Kirby-Bauer disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines [5].

Results

In our laboratory, we isolated 766 isolates from 1541 specimens sent over a period of seven months. Out of total, 1126 samples were from male patients and 415 from female patients. Mean age was 32.8 ± 10 years; range 14 years to 85 years.

Isolates	n (%)
S. aureus	88 (11.5)
Methicillin resistant S. aureus (MRSA)	97 (12.7)
Methicillin resistant S. epidermidis (MRSE)	50 (6.5)
Enterococcus faecalis	25 (3.2)
Enterococcus faecium	18 (2.3)
Vancomycin resistant Enterococcus	1 (0.13)
Corynebacterium spp.	6 (0.8)
Pyogenic Streptococci Gp (B,C,G)	5 (0.6)
Streptococcus pyogenes	3 (0.4)
Streptococcus pneumoniae	1 (0.13)
Total	**294 (38.4)**

Table 1: *Gram positive isolates.*

Organism	n (%)
Escherichia coli	162 (21.1)
Pseudomonas aeruginosa	115 (15)
Klebsiella pneumoniae	56 (7.3)
Acinetobacter baumannii	54 (7.1)
Proteus mirabilis	20 (2.6)
Enterobacter cloacae	12 (1.6)
Citrobacter freundii	12 (1.6)
Serratia marcescens	9 (1.2)
Serratia odorifera	8 (1.04)
Morganella morganii	7 (0.9)
Burkholderia cepacia	5 (0.6)
Providencia stuartii	4 (0.5)
Klebsiella oxytoca	4 (0.5)
Enterobacter aerogenes	2 (0.26)
Proteus vulgaris	1 (0.13)
Bacteroides fragilis	1 (0.13)
Total	**472 (61.6)**

Table 2: *Gram Negative isolates.*

	Resistant Isolates n (%)						
Antibiotic	**S. aureus n = 185**	**MRSE N = 50**	**E. faecalis N = 25**	**E. faecium N = 18**	**S. pyogenes N = 3**	**Strep spp n = 5**	**Coryneforms N = 6**
PEN	185 (100)	50 (100)	9 (36)	13 (72.2)	0 (0)	1 (20)	5 (83.3)
FOX	97 (52.4)	50 (100)					
COT	67 (36.2)	27 (54)					
DOX	28 (3.56)	22 (44)	13 (52)	4 (22.2)			
CIP	98 (53)	36 (72)	11 (44)	10 (55.5)	1 (33.3)	2 (40)	6 (0)
CLI	32 (17.29)	20 (40)			1 (33.3)	3 (60)	6 (0)
ERY	66 (35.67)	35 (70)	13 (52)	7 (38.9)	3 (100)	2 (40)	6 (0)
GM	84 (45.40)	31 (62)					
AK	26 (14.05)	14 (28)					
CAP	5 (2.7)	10 (20)	4 (16)	4 (22.2)			4 (66.6)
VAN	0 (0)	0 (0)	0 (0)	1 (5.55)	0 (0)	0 (0)	0 (0)
LNZ	1 (0.54)	1 (2)	0 (0)	0			0 (0)
TGC	12 (6.48)	3 6)					

Table 3: *Antibiotic susceptibility pattern of Gram positive organisms isolated from pus and pus swab samples.*
Abbreviations: *Pen(penicillin), Fox (cefoxitin), Cot (cotrimoxazole), Dox (doxycycline), Cip (ciprofloxacin), Cli (clindamycin), Ery (erythromycin), Gm (gentamicin), Ak (amikacin), Cap (chloramphenicol), Van (vancomycin), Lnz (linezolid), Tgc (tigecycline).*

Organism	Resistant Isolates n (%)															
	AMP	COT	CIP	AMC	CRO CAZ	IMP	MEM	GM	AK	TZP	SCF	ATM	FEP	DOX	MIN	TGC
E. coli N = 162	161 99.3	143 88.2	136 83.9	135 83.3	146 90.1	35 21.6	33 20.3	119 73.4	42 25.9	86 53	88 54.3					49 30.2
P. aeruginosa N = 116			77 66.3		86 74.1	36 31	35 30.1	85 73.2	71 61.2	57 49.1	65 56	107 92.2	76 67.8			
K. pneumoniae N = 56	56 100	50 89.2	49 87.5	50 89.2	51 91	21 37.5	24 42.8	46 82.1	32 57.1	34 60.7	32 57.1					41 73.2
A. baumannii N = 54	54 100	47 87	53 98.1		53 98.1	47 87		50 92.5	42 77.7	46 85.1	43 79.6		49 90.7	40 74	37 68.5	35 64.8
P. mirabilis N = 20	20 100	20 100	19 95	16 80	18 90	1 5	1 5	16 80	3 15	5 25	4 20			20 100		20 100
C. freundii N = 12	12 100	8 66.6	10 83.3	12 100	12 100	5 41.6	5 41.6	7 58.3	5 41.6	5 41.6	6 50			10 83.3		6 50
E. cloacae N = 12	12 100	11 91.6	10 83.3	12 100	11 91.6	7 58.3	7 58.3	9 75	8 66.6	7 58.3	8 66.6			7 58.3		9 75
S. marcescens N = 9	9 100	9 100	9 100	9 100	9 100	7 77.7	7 77.7	9 100	9 100	7	7					9 100
S. odorifera N = 8	8 100	7 87.5	7 87.5	8 100	8 100	2 25	2 25	5 62.5	2 25	5 62.5	4 50			6 75		3 37.5
M. morganii N = 7	7 100	7 100	7 100	7 100	7 100	1 14.2	0	6 85.7	4 57.1	0	2 28.5					3 42.8
B. cepacia N = 5		0		3 60		1 20	0									
P. stuartii N = 4	4 100	4 100	4 100		4 100	0	0	0	3 75	1 25	2 50			4 100		4 100
K. oxytoca N = 4	4 100	0	0	2 50	0	2 50	0	2 50	2 50	2 50	2 50			0		1 25
E. aerogenes N = 2	2 100	1 50	1 50	2 100	1 50	0	0	2 100	2 100	1 50	1 50			2 100		2 100
P. vulgaris N = 1	1 100	1 100	1 100	1 100	0	0	0	0	0	1 100	1 100					1

Table 4: *Antibiotic susceptibility pattern of Gram negative organisms isolated from pus and pus swab specimen.*
Abbreviations: *Amp(ampicillin), Cot(cotrimoxazole), Cip(ciprofloxacin), Amc(amoxicillin-clavulanicacid), Cro(ceftriaxone), Caz(ceftazidime), Imp(imipenem), Mem(meropenem), Gm(gentamicin), Ak(amikacin), Tzp(tazobact-ampiperacillin), SCF(Cefoperazone-sulbactam), Atm(aztreonam), Fep(cefipime), Dox(doxycycline), Min(minocycline), Tgc(tigecycline)*

Gram positive isolates constituted 294 (38.4%) of the total 766 isolates (Table 1). *S. aureus* was the most predominant pathogen, isolated from 185 (24.2%) samples and among these 97(12.7%) were MRSA. Other significant Gram positive organisms were *Enterococcus faecalis* 25 (3.2%), *Enterococcus faecium* 18 (2.3%), *Corynebacterium* species 6 (0.8%), *pyogenic Streptococci* belonging to Lancefield Groups B, C and G 5 (0.6%), *Streptococcus pyogenes* 3 (0.6%) and single isolate of *Streptococcus pneumoniae* (0.13%).

Total GNB isolated were 472 (61.6%).The most frequent isolate was *Escherichia coli* 162 (21.14%) followed by *Pseudomonas aeruginosa* 115 (15%), *Klebsiella pneumonia* 56 (7.31%), *Acinetobacter baumannii* 54 (7.15%), *Proteus mirabilis* 20 (2.6%), *Enterobacter cloacae* 12 (1.6%), *Citrobacter freundii* 12 (1.6%). Details are given in table 2. Antimicrobial susceptibilities are given in table 3 for Gram positive organisms and table 4 for Gram negative isolates.

Discussion

SSIs are a common problem worldwide with infection rates ranging from 20% to 76.9%. The disparity in results signified lack of awareness and training in infection control practices in developing world leading to unacceptably high infection rates [3]. Data on infection rates following surgical procedures is scarce in developing countries due to lack of clinical audits in most of the hospitals. Khan., *et al*. have reported 9.29% incidence of infection following surgical procedures from Peshawar Pakistan [6]. Shahane., *et al*. from India reported SSI incidence of 6% [6]. Infection rate in patients undergoing surgical procedures at Aga Khan Hospital in Kenya was 7% [7].

Nearly all centers are reporting increase in multidrug resistant Gram positive and negative organisms; the trend of bacterial isolates and their sensitivity varies from country to country. Review of literature has shown that Gram negative organisms causing infections in surgical wards are more prevalent than Gram positive isolates. A nationwide surveillance study conducted in Japan revealed that GNB were responsible for majority of infections in surgical setups [8]. The most common Gram negative isolate was *Escherichia coli* followed by *Pseudomonas aeruginosa* and *Enterobacter cloacae*. *Enterococci* were the most common Gram positive isolates followed by Streptococci and Staphylococci. Study conducted by Ali., *et al*. [8] at Hyderabad, stated *E. coli* (60.7%) as the leading cause of SSI followed by Klebsiella (20.5%), *S. aureus* (9.8%), and *Pseudomonas aeruginosa* (4%) [9]. *S. epidermidis* was not a significant isolate in their study, while we isolated methicillin resistant *S. epidermidis* in 6.5% of positive samples. We also detected *S. aureus* as the most prevalent Gram positive isolate in our setup. The overall increase in Gram negative isolates compared to Gram positive isolates share the same trend as reported earlier.

Study conducted in Bangladesh by Haque., *et al* reported high prevalence of resistance to gentamicin, ciprofloxacin, erythromycin and doxycycline in MRSE isolate however none of the isolate was found resistant to vancomycin and rifampicin [10]. In our study, 6.5% of Coagulase negative Staphylococcal isolates was methicillin resistant and all isolates were sensitive to vancomycin with one linezolid resistant isolate.

MRSA is an important pathogen in surgical wards of hospitals across the globe. MRSA constituted 12.7% of total isolates in our study with resistant to multiple groups of antibiotics. Similar findings were reported by Rahman., *et al*. in Peshawar [11]. In a multicentre study conducted at four tertiary care hospitals of Lahore by Bukhari., *et al*. 22% of infections in surgical units at four tertiary care hospitals were attributed to MRSA [12]. Additionally, MRSA isolates in the study were more resistant to other tested antibiotics as compared to our isolates. Comparable results of hospital acquired infections by MRSA and their susceptibility pattern were reported by Aghazadeh., *et al*. from Iran and Al-Talib., *et al*. from Malaysia [13,14].

Gram negative isolates with resistance to multiple classes of antibiotics are responsible for large number of surgical wound infections [15]. The alarming trend of escalating Gram negative infections in surgical wounds was also observed by Ali., *et al*. [9]. They reported high resistance against fluoroquinolones among *E. coli* and Klebsiella spp. similar to our study. Maraki., *el al*. from Greece reported *E. coli*, Pseudomonas aeruginosa and *Acinetobacter baumannii* as the most common Gram negative isolates from surgical wards, similar to our study [16]. A similar observation was reported by Gadebo., *et al*. from Ethiopia [17]. Susceptibility of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates against aminoglycosides and carbapenems were higher in comparison with our results in another study from India [18]. A nationwide survey conducted by Takesue., *et al*. in Japan on isolates from surgical wound infections revealed a very high prevalence of MRSA 72% among *S. aureus* isolates, however very low level of resistance was reported in Gram negative isolates contrasting our results. Majority of isolates were susceptible to carbapenems and aminoglycosides and tazobactam-piperacillin although resistance to fluoroquinolones was more common [8]. Shahane., *et al*. from India also reported predominance of

Gram negative isolates from surgical site infections with *E. coli* (31%) as the most common isolate followed by *Pseudomonas aeruginosa* (25%). Amikacin and gentamicin were the most effective drugs against Gram negative isolates; a very high level of resistance to fluoroquinolones and third generation cephalosporins was reported similar to our study [3]. Similar rise in resistance to antibiotics among *E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Acinetobacter baumannii* was also reported from Lebanon [19].

Conclusion

The trend in SSIs is shifting towards Gram negative isolates with predominant *Escherichia coli* followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii. S. aureus* dominates among Gram positive pathogen. Under the existing antimicrobial resistance pattern, the best way to effectively control spread and emergence of these problem bugs is adherence to good infection control practices and antibiotic stewardship.

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