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Research Article

EMERGING MULTIDRUG RESISTANCE AND EXTENSIVE DRUG RESISTANCE IN BACTERIAL PATHOGENS ISOLATED FROM PUS SAMPLES AT A TERTIARY CARE INSTITUTE OF KASHMIR, INDIA

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Abstract- Background: The resistance to antibiotics is increasing at a pace faster than can be controlled. The most apparent reason is the in appropriate use of antibiotics. Multidrug resistant (MDR) and extensively drug resistant (XDR) organisms are an important cause of hospital-acquired infections, creating a therapeutic challenge. Data regarding such organisms is not available. Therefore, the study was done to identify various organisms and their antimicrobial sensitivity patterns from pus samples, thus providing data about MDR and XDR organisms in our institute and guiding the appropriate use of antibiotics to prevent the emergence of such organisms. **Methodology:** 501 pus aspirates were studied over a period of 6 months for identification and antibiotic sensitivity. **Results:** 200 (40%) samples were culture positive and aerobic Gram-positive cocci showed predominance with a total of 110 (52.6%) isolates. 99 (47.4%) isolates were aerobic Gram-negative bacilli. The most common isolate was *Staphylococcus aureus* [79 (37.8%)]. Gram-positive organisms showed higher resistance towards ampicillin, amoxicillin-clavulanate, and quinolones. Gram negatives organisms showed more resistance towards quinolones (55 to 84%) but were highly sensitive to carbapenems and polymyxin B. 111 (53.1%) isolates were MDR and 19 (9.1%) isolates were XDR. **Conclusion:** The resistance spectrum of pathogens varies in different regions. Therefore, local resistance patterns have to be known for appropriate antimicrobial use. In our study, a significant proportion of MDR along with some XDR organisms was seen. Urgent steps should be taken to minimize any resistance resulting due to inappropriate use of antibiotics, and identification of the causative pathogen before beginning therapy should be done.

Keywords- pus, isolates, antibiotics, multidrug resistant, extensively drug resistant.

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Introduction

Infections of human skin and soft tissues due to microbial pathogens as a result of trauma, burn injuries or surgical procedures lead to the production of pus, a white to yellow fluid comprised of dead WBCs, cellular debris, and necrotic tissue [1]. Aerobic as well as anaerobic bacteria have been implicated in such infections which commonly occur under hospital environment and result in significant morbidity, prolonged hospitalization, and huge economic burden [2]. The probability of these infections largely depends on local wound conditions, microbial burden and the host defense mechanisms. Effective treatment of such wound infections depends upon the proper understanding of causative pathogen, pathophysiology of the infectious process and pharmacology of the therapeutic agents [3].

Antibiotic resistance is a serious problem that has the potential to drag the world into the pre-antibiotic era. [4] Because of the irrational use of antibiotics, virulent strains adapt to the environment and it is a concern to the healthcare services. The antibiotic pipeline has also become dry and it is the need of the hour to reserve antibiotics like carbapenems to multidrug-resistant organisms [5]. These organisms continue to be an important cause of hospital-acquired infection and pose a therapeutic challenge [3].

The issue of using inappropriate antibiotics stems primarily from the inherent inclination of doctors toward prescribing the potent antibiotics. As one expert puts it, "*When it comes to prescribing antibiotics, most doctors use the canon when a gun can be used to kill the same enemy*" [4]. Keeping in view that the prevalence of multidrug, extensive and pan drug resistant organisms is increasing at a faster pace throughout the world and no such study had been conducted in our institute, an understanding of antimicrobial resistance in our hospital is an urgent need in order to develop proper infection control policies. The study was conducted to isolate the various organisms from pus samples and to study their susceptibility pattern, thus guiding the appropriate use of antibiotics which can further prevent the emergence of multidrug and extensively drug-resistant organisms.

Materials and Methods

This was a cross-sectional study conducted in the Department of Microbiology, Sher-i-Kashmir Institute of Medical Sciences, a tertiary care hospital in Kashmir, India. A total of 501 pus samples were studied over a period of 6 months from July 2017 to December 2017. Pus aspirates were collected by using sterile disposable syringes and sent immediately to the bacteriology section of microbiology laboratory, as aspirates of pus taken with a syringe are more readily protected and may be more reliable. Samples were inoculated on to blood agar, Mac Conkey Agar, Robertson Cooked Meat (RCM) broth and these were incubated aerobically at 37°C for 24 to 48 hrs. Identification of isolates from positive cultures was done using standard microbiological techniques [6]. Antibiotic sensitivity testing of all isolates was done by Kirby Bauer's disc diffusion method on Muller Hinton agar and results were interpreted as per CLSI guidelines. [7] Standard antibiotic discs of amikacin (30mcg), amoxycillin-clavulanate (20/10mcg), ampicillin (30mcg), cefoperazone-sulbactam (75mcg/10mcg), cefoxitin (30mcg), -

ciprofloxacin (5mcg), levofloxacin, clindamycin (2mcg), polymixin B (10mcg), cotrimoxazole (25mcg), erythromycin (15mcg), gentamicin (10mcg), imipenem (10mcg), linezolid (30mcg), piperacillin-tazobactam (100/10mcg), cefoperazone-sulbactam (100/10mcg), vancomycin (30mcg), ceftazidime (30mcg), tobramycin (30mcg), ticarcillin-clavulanate (100/10mcg), carbenicillin (10mcg), and aztreonam (30mcg) were tested. All the culture media, biochemical media and antibiotic discs used were obtained from Hi Media laboratories, India.

Results

Of the total 501 pus samples, 200 (40%) were culture positive among which 9(4.5%) samples showed polymicrobial growth with two organisms. The total numbers of isolates recovered were 209 and among them aerobic Gram-positive cocci showed predominance with a total of 110(52.6%) isolates. 99(47.4%) isolates were Gram-negative bacilli. The most common pathogen isolated was *Staphylococcus aureus* (79,37.8%) followed by *Escherichia coli* (56, 26.8%) as shown in [Table-1]. The sensitivity patterns of Gram-positive and Gram-negative pathogens are listed in [Table-2 and 3] respectively. 53.1% (111) isolates were multidrug resistant showing resistance to at least three groups of antibiotics and 19(9.1%) isolates were extensively drug resistant(XDR) showing resistance to at least one drug in all groups of antibiotics except for one or two groups [Table-4].

Table-1 *Distribution of organisms isolated*

Serial No	Organism	N(%)
1.	*Staphylococcus aureus*	79 (37.3)
2.	*Escherichia coli*	56 (26.7)
3.	CoNS	23 (11.0)
4.	*Klebsiella pneumoniae*	17 (8.1)
5.	*Acinetobacter* spp	16 (7.6)
6.	*Pseudomonas* spp	9 (4.3)
7.	*Enterococcus* spp	8 (3.8)
8.	*Proteus* spp	1 (0.47)
	Total	**209**
N=Number %=Percent		

Table-2 *Antibiogram of Gram positive Bacteria.*

Antibiotic	*Staphylococcus aureus* N(%)		CoNS N (%)		*Enterococcus* spp. N(%)	
	S	R	S	R	S	R
Ampicillin	0 (0)	79 (100)	0 (0)	23 (100)	1 (12.5)	7 (87.5)
Amoxicillin-clavulanate	22 (27.8)	57 (72.1)	11 (47.8)	12 (52.1)	1 (12.5)	7 (87.5)
Cefoxitin	22 (27.8)	57 (72.1)	11(47.8)	12 (52.1)	-	-
Erythromycin	32 (40.5)	47(59.4)	18 (78.2)	5 (21.7)	-	-
Clindamycin	59 (74.6)	20 (25.3)	18 (78.2)	5 (21.7)	-	-
Ciprofloxacin	22 (27.8)	57 (72.1)	8 (34.7)	15 (65.2)	1(12.5)	7 (87.5)
Levofloxacin	28 (35.4)	51 (64.5)	11(47.8)	12 (52.1)	2 (25)	6 (75)
Co-trimoxazole	29 (36.7)	50 (63.2)	14 (60.8)	9 (39.1)	-	-
Linezolid	79 (100)	0 (0)	23 (100)	0 (0)	8 (100)	0 (0)
Vancomycin	79 (100)	0 (0)	23 (100)	0 (0)	5 (62.5)	3 (37.5)
S= Sensitive R= Resistant N=Number %=Percent						

Table-3*Antibiogram of Gram Negative Bacteria.*

Antibiotic	*Escherichia coli* N(%)		*Klebsiella pneumoniae* N (%)		*Proteus vulgaris* N (%)		*Pseudomonas* spp N (%)		*Acinetobacter* spp N (%)	
	S	R	S	R	S	R	S	R	S	R
Amikacin	48 (85.7)	8 (14.20)	11 (64.7)	6 (35.2)	1 (100)	0 (0)	5 (55.5)	4 (44.4)	7 (43.7)	9 (56.2)
Gentamicin	37 (66.0)	19 (34)	8 (47.0)	9 (52.9)	1 (100)	0 (0)	3 (33.3)	6 (66.6)	5 (31.2)	11 (68.7)
Tetracycline	30 (53.6)	26 (46.4)	9 (52.90	8 (47)	1 (100)	0 (0)	2 (22.2)	7 (77.8)	7 (43.7)	9 (56.2)
Polymixin B	56 (100)	0 (0)	17 (100)	0 (0)	0 (0)	1 (100)	9 (100)	0 (0)	16 (100)	0 (0)
Ciprofloxacin	9 (16)	47 (84)	4 (23.5)	13 (76.4)	1 (100)	0 (0)	4 (44.5)	5 (55.5)	3 (18.7)	13 (81.3)
Levofloxacin	18 (32.1)	38 (67.8)	6 (35.3)	11 (64.7)	1 (100)	0 (0)	4 (44.5)	5 (55.5)	4 (25)	12 (75)
Piperacillin tazobactam	26 (46.4)	30 (53.5)	8 (47)	9 (53)	1 (100)	0 (0)	4 (44.5)	5 (55.5)	5 (31.2)	11(68.7)
Cefoperazone sulbactam	23 (31.0)	33 (59)	5 (29.4)	12 (70.5)	1 (100)	0 (0)	4 (44.5)	5 (55.5)	7 (43.7)	9 (56.2)
Imipenem	48 (85.7)	8 (14.3)	13 (76.4)	4 (23.5)	1 (100)	0 (0)	6 (66.6)	3 (33.4)	9 (56.2)	7 (43.7)
Meropenem	25 (44.6)	31 (55.3)	10 (58.8)	7 (41.2)	1 (100)	0 (0)	4 (44.5)	5 (55.5)	6 (37.5)	10 (62.7)
Ceftazidime	-	-	-	-	-	-	1 (11.1)	8 (88.9)	-	-
Tobramycin	-	-	-	-	-	-	4 (44.5)	5 (55.5)	-	-
Ticarcillin clavulanate	-	-	-	-	-	-	0 (0)	9 (100)	-	-
Carbenicillin	-	-	-	-	-	-	3 (33.4)	6 (66.6)	-	-
Aztreonam	-	-	-	-	-	-	2 (22.2)	7 (77.8)	-	-
S= Sensitive R= Resistant N=Number %=Percent										

Table-4 *Multidrug and extensive drug resistance pattern of isolates.*

Organism	Total N(%)	MDR N(%)	XDR N(%)
Staphylococcus aureus	79(37.3)	57(72.1)	0
CoNS	23(11.0)	13(56.5)	0
Enterococcus spp	8(3.8)	5(62.5)	0
Escherichia coli	56(26.7)	16(28.5)	4(7.1)
Acinetobacter spp	16(7.6)	8(50)	8(50)
Pseudomonas spp	9(4.3)	4(44.4)	4(44.4)
Proteus spp	1(0.47)	0(0)	0
Klebsiella pneumoniae	17(8.1)	8(47)	3(17.6)
Total	**209(100)**	**111(53.1)**	**19(9.1)**

Discussion

Resistance to antimicrobials is one of the most important public health problems particularly in developing countries where easy accessibility and high consumption of medicines have led to the inappropriate use of antibiotics along with greater levels of resistance [8]. Of the 501 pus samples collected from patients attending to our hospital, 200 (39.9.%) samples showed bacterial growth after 24–48 hours of incubation whereas 301 samples (60.1%)were negative for growth. A total of 209 isolates were recovered and majority of them were aerobic Gram-positive cocci 110(52. 6%). Gram-negative organisms isolated were 99(47.4%). Similarly, Muluye, *et al.*, isolated more Gram-positive organisms than Gram-negative isolates in their study [9].

The predominant isolate in our study was *Staphylococcus aureus* [79 (37.3%)] followed by *Escherichia coli* [56(26.7%)], CoNS [23(11.0)], *Klebsiella pneumoniae* [17(8.1%)], *Acinetobacter* spp. [16(7.6%)] and *Pseudomonas* spp [9(4.3%)]. Different studies show that the most common pyogenic bacteria isolated include Gram-positive cocci like *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Enterococci* and Gram-negative bacilli like *Escherichia coli*, *Klebsiella pneumoniae, Proteus,* and *Pseudomonas* [10,11]. *Staphylococcus* has been reported as the predominant organism in other studies also [12]. The possible reason for the high frequency of this microorganism is that majority of these isolates are present as normal flora on the skin and in the gut of healthy individuals. When there is a breach on skin and soft tissues, they get displaced to other sterile sites and disseminate easily. Moreover, most of these bacteria are commonly found in the hospital environment which might increase the proportion of infections due to these organisms and cross-contamination among admitted patients [13].

In our study, Gram-positive isolates were least sensitive to penicillins and most susceptible to vancomycin and linezolid. Gram-negative organisms showed the highest resistance towards quinolones and cephalosporins with moderate sensitivity to aminoglycosides and highest sensitivity to polymyxin B followed by imipenem. These findings correlate with several studies [1,10,14].

Antimicrobial resistance is not only increasing the healthcare costs but also the severity and death rates from certain infections that could have been avoided by prudent use of the existing and newer antimicrobial agents. Rational use of antimicrobials is possible by forming local, national and global wide antibiogram [15].

In this study, we isolated 53.1% MDR organisms which were resistant to more than two antibiotic groups. Among the Gram-positive organism's prevalence of MDR isolates was 68.1% with the highest resistance shown by *Staphylococcus aureus* (72.1%) followed by *Enterococcus* spp (62.5%) and coagulase-negative *staphylococci* (56.5%). Similar results had been reported by Bhandari, *et al*., who reported 66% MDR organisms from pus isolates in their study, among which 55.2% of Gram-positive isolates were MDR [16]. *Staphylococcus aureus* was the most common Gram-positive cocci and MRSA accounted for 56.25% of total staphylococcal isolates in their study [16]. In our study, the prevalence of MDR Gram negative isolates was seen to be 36.3% with the highest resistance shown by isolates of *Acinetobacter* spp. (50%), followed by *Klebsiella pneumoniae* (47%), *Pseudomonas* spp. (44.4%) and *Escherichia coli* (28.5%). Also reports from various studies from hospitals in India suggest that the prevalence of MDR Gram negative isolates ranges between 19% and 60% [17]. In another study by Goel, *et al.,* the prevalence of MDR Gram-negative isolates was estimated to be 70% [18].

We isolated 9.1% XDR organisms and all of them were Gram negative. *Acinetobacter* spp. (50%) was the commonest XDR organism. Gram positive isolates did not show any extensive drug resistance in our study. Basak, *et al*., in their study isolated 13.8% XDR organisms from different clinical specimens while detecting the incidence of MDR and XDR organisms [19]. Pan-drug resistance was not seen in any of the isolates in our study.

Inappropriate use of antibiotics can result in the high use of reserved drugs like meropenem and colistin in the hospital which causes the selection of resistant isolates that can survive and spread. Increased incidence of drug resistant strains observed in our study may be also attributed to the fact that our hospital is a tertiary care centre. Patients from various remote areas are admitted for treatment but before attending the hospital, most of the patients have already taken antibiotics as advised by medical practitioners or from over-the-counter sale of antibiotics often in improper dose.

Conclusion

Bacterial isolates exhibited high to moderate levels of resistance against different classes of antibiotics. Unknown susceptibility pattern of bacterial isolates encourages the empirical selection of broad-spectrum antibiotics. Urgent measures are required not only to minimize the use of antimicrobials for prophylactic and therapeutic purposes but also to look for alternative strategies for the control of such bacterial infections. Strict health policies should be implemented to restrict the purchase, prescription and unsupervised antibiotic use as well as for continuous monitoring and reporting of antibiotic resistance.

Application of research: The above study will guide clinicians in our hospital to properly prescribe the antibiotics and help a step forward towards antibiotic stewardship.

Research Category: Clinical microbiology

Abbreviations: MDR-multidrug resistant
XDR-extensive drug resistant
MRSA-methicillin resistant staphylococcus aureus
CoNS-coagulase negative stapyhlococcus

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