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Antibacterial resistance pattern in isolates from pus samples: An observational study

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Abstract

Development of antibacterial drug resistance has made the cure of bacterial wound infections very challenging, especially in post-operative cases. Current study was carried out at Ayub Teaching Hospital, Abbottabad. The aim of this study was to identify organisms developing resistance and to detect the predominant bacterial sketch and its antibiogram in Abbottabad. An observational study was directed for a period of 1 year from June 2016 to May 2017 at Ayub Teaching Hospital, Abbottabad**.** Pus samples from several locations were collected aseptically from 300 patients and were subjected to isolation and identification of aerobic bacteria by standard procedure. Out of the 300 clinical samples, 238 showed bacterial growth. *Staphylococcus aureus* was the most common organism isolated (57.5%), followed by *Escherichia coli* (19.72%), *Klebsiella* (12.28%), *Pseudomonas* (10.50%) among others. *S. aureus* was found to be highly resistant to penicillin, ampicillin, amoxicillin and nalidixic acid, while being sensitive to sipraxin and vancomycin. On the other hand, of the gram-negative bacilli isolated, *E. coli* was found to be more common, followed by *Klebsiella*, *Pseudomonas*, *Proteus*, and *Acinetobacter*. They were all found to be highly resistant to ciprofloxacin and amoxicillin and fairly sensitive to amikacin and azaetronam. This study shows that in spite of the geographical diversity, the infecting bacterial isolates and their antibiogram from this part are found to be alike to those found in any other part of Pakistan.

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Introduction

The human outer covering (skin) and soft tissue infections caused by microbial pathogens during or after trauma, burn injuries, and surgical procedures consequence in the assembly of pus, a white to yellow fluid comprised of deceased WBCs, cellular fragments, and necrotic tissues (Cogen *et al*., 2008; Dryden, 2010; Scalise *et al*., 2015). Both aerobic and anaerobic bacteria have been implicated in wound infections which frequently occur under hospital atmosphere and result in significant morbidity, extended hospitalization, and enormous financial load (Bowler *et al*., 2001). The development of antibiotic resistance and its quick spread among pathogenic bacterial isolates are considered as serious threats to the public health worldwide (Prestinaci *et al*., 2015). During the last few decades, multidrug-resistant Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacterial strains such as *Acinetobacter baumannii, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, were progressively associated with pus infections under hospital settings due to inadequate dose regimen of antibiotics (Iredell *et al*., 2016; Misic *et al*., 2014; Rice, 2006). Rapid development of multidrug-resistant bacteria poses a grave threat to public health globally due to the limited treatment choices (Cerceo *et al*., 2016; Iredell *et al*., 2016). Various studies across the globe have been consistent enough to show a predictable bacterial profile in pyogenic wound infections.

A cross-sectional study designed to determine the distribution of the bacterial pathogens and their antimicrobial susceptibility from suspected cases of post-operative wound infections, also revealed *Staphylococcus aureus* (63%) was the most frequently isolated pathogenic bacteria, followed by *Escherichia coli* (12%), *Pseudomonas* species (9.5%), *Klebsiella* species (5%), *Proteus* species (3.5%) and coagulase negative. *Staphylococcus* study on microbiological profile of diabetic foot ulcers and its antibiotic susceptibility pattern in a teaching hospital in Gujarat, revealed that *Pseudomonas aeruginosa* (27%) was the most common isolate causing diabetic foot infections followed by *Klebsiella* species (22%), *Escherichia coli* (19%), *Staphylococcus aureus* (17%), *Proteus* species (7%), *Enterococci* (3%), *Acinetobacter* (2%), CoNS (2%) and *Providencia* (1%) (Mehta *et al.,* 2014).

A study done in a tertiary hospital, Pakistan on burn wounds, revealed *Staphylococcus aureus* (57.98%) to be the most causative organism in burn wound infections followed by *Pseudomonas aeruginosa* (19.33%), *Klebsiella pneumonia* (8.4%), *Proteus* species (4.2%), *Staphylococcus epidermidis* (3.36%), *Escherichia coli* and *Enterobacter* (2.52%) each, *Citrobacter* and *Serratia* (0.84%) each (Ahmed *et al.,* 2013). Though a study done in Ibadan, Nigeria on burn wound infections revealed *Klebsiella* species to be the most commonly isolated pathogen, constituting (34.4%), closely followed by *Pseudomonas aeruginosa* (29.0%) and *Staphylococcus aureus* (26.8%) (Kehinde *et al.,* 2004).

In a two-year period, study done on bacterial profile of burn wounds infections at a burn unit Nishtar hospital Multan, the frequency of gram negative organisms was found to be high with *Pseudomonas aeruginosa* (54.4%) being the most common isolate, followed by *Staphylococcus aureus* (22%), *Klebsiella* species (8.88%), *Staphylococcus epidermidis* (5.79%), *Acinetobacter* species (4.63%), *Proteus* species (2.70%) and *Escherichia coli* (1.54%) (Shahzad *et al.,* 2012). Even though gram negative bacteria increased significantly but still *Staphylococcus aureus* was being continued as a major etiological agent of pyogenic infections species (3.5%) (Shriyan *et al.,* 2010).

The objective of this study is to characterize the pyogenic bacteria from pus samples and to determine their antibiotic susceptibilities to numerous generations of antibiotics frequently used in treatment.

Materials and methods

A total of 300 pus samples were collected by sterile syringe aspiration and by sterile swabs from inpatients and outpatients over a period of 1 year from June 2016 to May 2017 in accordance with standard protocols and ethical guidelines.

Pus samples were collected from skin (furuncles, pustules, and abrasions), post operated wounds, ears, and legs. Pus samples were kept in Cary-Blair transport medium until processed for Gram staining and culturing. The samples were aseptically inoculated on blood agar (with 7% sheep blood) and MacConkey agar plates, incubated aerobically at 35°C–37°C for 24–48 hours. Identification and characterization of isolates were performed on the basis of Gram staining, microscopic characteristics, colony characteristic, and biochemical tests using standard microbiological methods.

Antibiotics susceptibility testing

Antibiotic susceptibilities of bacterial isolates were determined according to the method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2010). Briefly, inocula were prepared for each bacterial isolate by adjusting the turbidity to 0.5 McFarland standard and spread on Muller-Hinton agar plates. Antibiotics discs containing amikacin (30µg), amoxicillin-clavulanic acid (30µg), aztreonam (30µg), ampicillin (10µg), azithromycin (30µg), cefepime (30µg), Cefoperazone/ Sulbactam (75/30µg), ceftriaxone (30µg), cefotaxime (30µg), cefuroxime (30µg), ciprofloxacin (1µg), clindamycin (2µg), cloxacillin (30µg), trimethoprim/ sulfamethoxazole (25µg), erythromycin (15µg), gentamicin (10µg), imipenem (10µg), levofloxacin (5µg), linezolid (30µg), meropenem (10µg), ofloxacin (5µg), piperacillin-tazobactam (100/10µg), tetracycline (30µg), and vancomycin (30µg) Oxacillin Enozabid, sparfloxacin, Moxifloxacin, nitrofurontion, nalixidic acid, cephrodine and cefixime were placed on the agar plates and incubated overnight at 37°C for 24 h. The zones of inhibition were measured and the isolates were classified as sensitive, intermediate, and resistant according to CLSI tables and guidelines (CLSI, 2010).

Result and discussion

Out of 300 pus samples collected from different wards of the hospital, 238 samples showed bacterial growth after 24–48 hours of incubation. Based on Gram staining, morphological features, culture characteristics, and biochemical characterization, the bacterial isolates were assigned to seven bacterial species. *Staphylococcus aureus* was the most common organism isolated (57.5%), followed by *Escherichia coli* (19.72%), *Klebsiella* (12.28%), *Pseudomonas* (10.50%) as shown in fig. 1. Our findings correlate with Zhang *et al.*, (2014) who reported predominance of *E. coli, S. aureus, K. pneumoniae*, and *P. aeruginosa* in pus samples from patients with severe intra-abdominal infection. In another study, *S. aureus* was the dominant bacterial species from wounds followed by *P. aeruginosa, P. mirabilis, E. coli*, and *Corynebacterium* spp. (Bessa *et al.*, 2015). According to Dryden, (2009) *S. aureus* and MRSA are major cause of soft tissue infections in hospitalized patients. Several other reports have also implicated *Pseudomonas, Staphylococcus, Streptococcus, Klebsiella,* and *E. coli* in wound infections (Lockhart *et al.*, 2007; Misic *et al.*, 2014). As showed in Table 1, *Staph. aureus* was found to be highly resistant to ampicillin (98.30%), amoxicillin (96.40%) and nalidxicacid (98.10%). while being sensitive to sparfloxacin (51.05%) and vancomycin (50%). On the other hand, among gram-negative bacilli isolated, *E. coli* was found to be more common, followed by *Klebsiella*, *Pseudomonas*, *Proteus*, and *Acinetobacter*. They were all found to be highly resistant to ciprofloxacin (100%) and amoxicillin (100%) and fairly sensitive to amikacin (69.79%) azaetronam (51%) Imipenem (70.23%) Nitrofurontion (63.45%) Enoxabid (65.21%) This study shows that in spite of the geographical diversity, the infecting bacterial isolates and their antibiogram from this part are found to be alike to those found in any other part of Pakistan.

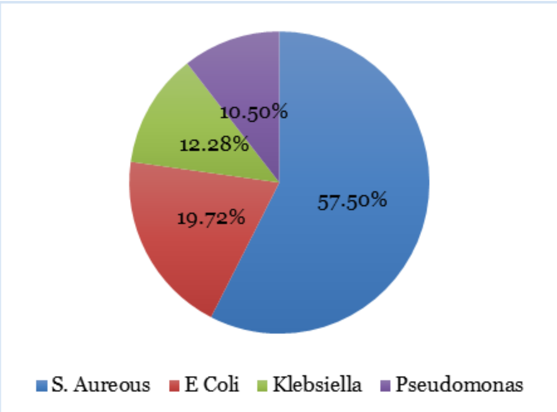

Fig. 1. Most common isolated bacterial organism.

Table 1. Susceptibility pattern of different pathogens isolated from pus samples to various antimicrobial agents.

Antibiotics	*S.aureus*		*Pseudomonas*		*E. coli*		*Klebsiella*		*Proteous*		*E.facecalis*		Actinomycetes	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin+ Sulbactum	1.70%	98.30%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%
Amoxicillin	3.60%	96.40%	0%	100%	2%	98%	21%	79%	0%	100%	0%	100%	0%	100%
Cloxacillin	36.43%	63.57%	_	_	_	_	_	_	_	_	_	_	0%	100%
Oxacillin	17.82%	82.18%	_	_	_	_	_	_	_	_	100%	0%	0%	100%
Nezkil	11%	89%	0%	100%	0%	100%	48%	52%	0%	100%	0%	100%	_	_
Pipracillin+ sulbactum	17.89%	82.11%	24%	76%	0%	100%	_	_	_	_	_	_	100%	0%
Ciprofloxacin	50.5%	49.5%	32%	68%	11.62%	88.38%	24.13%	75.87%	0%	100%	0%	100%	0%	100%
Ofloxacin	17.52%	82.48%	28%	72%	25.58%	74.42%	41.38%	58.62%	50%	50%	0%	100%	0%	100%
Gentamycin	41.90%	58.10%	68%	32%	44%	56%	41%	59%	50%	50%	0%	100%	_	_
Amikacin	22.5%	77.50%	84%	16%	69.79%	30.21%	65.51%	34.49%	0%	100%	0%	100%	0%	100%
Spraxin	51.05%	48.95%	32%	68%	30%	70%	28%	72%	0%	100%	0%	100%	_	_
Cefhradine	30.46%	69.54%	_	_	18.60%	81.40%	24%	76%	0%	100%	100%	0%	0%	100%
Ceftazidime	41.92%	58.08%	_	_	18.60%	81.40%	_	_	0%	100%	0%	100%	_	_
Doxycyclin	42.97%	57.03%	_	_	_	_	_	_	_	_	100%	0%	_	_
Tetracycline	27.70%	72.30%	_	_	_	_	30%	70%	0%	100%	0%	100%	0%	100%
Nalidixic acid	1.90%	98.10%	_	_	_	_	_	_	_	_	_	_	0%	100%
Cefixime	_	_	28%	72%		_	24%	76%	0%	100%	0%	100%	_	_
Cefoperazone	_	_	32%	68%	14%	86%	_	_	_	_	0%	100%	_	_
Azetronem	_	_	52%	48%	51%	49%	28%	72%	0%	100%	0%	100%	_	_

Conclusion

In conclusion, pyogenic wound infections were found prevalent in Ayub teaching hospital and *S. aureus* isolates showed highest incidence followed by *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Proteous*. *Actinomycetes*, *E. faecalis* spp. Bacterial isolates exhibited high to moderate levels of resistance against different classes of antibiotics. The susceptibility data from this report may be worth consideration while implementing empiric treatment strategies for pyogenic infections. At the same time, strict health policies should also be implemented to regulate the purchase and prescription also restrict the unsupervised antibiotic use as well as continuous monitoring and reporting antibiotic resistance.

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