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PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF AEROBIC BACTERIAL ISOLATES FROM PUS/WOUND SWAB SAMPLES IN A TERTIARY CARE HOSPITAL, KATHMANDU.

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ABSTRACT

The organisms responsible for producing infection in different anatomical sites and their antimicrobial patterns show a wide range of variations from one place to another. The difficulty in therapeutic challenges due to increased incidence of drug resistance in organisms has been associated with poor patient outcomes. During Feb 2015 to Jan 2016, a total of 1052 pus samples (collected by swab or aspiration) from different anatomical sites received in microbiology laboratory of Nepal Medical College and Teaching Hospital (NMCTH) were included in this study. Samples were processed for isolation of organisms, their identification was done by standard microbiological techniques followed by antimicrobial susceptibility test using Kirby Bauer disc diffusion method. Vancomycin screening agar was used for Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. Of the 1052 samples processed, 502 had microbial growth of which, 491 yielded single organism, 7 yielded more then one organism and 4 grew Candida albicans. The most common bacterial isolate was *Staphylococcus aureus* (n=211), 9.96% of which being MRSA, none were resistant to vancomycin. The other organisms isolated were *Escherichia coli* (n=116), *Acinetobacter calcoaceticus- Acinetobacter baumannii* (ACB) complex (n=56), *Klebsiella pneumoniae* (n=32), Coagulase Negative Staphylococcus (n=27) and others. Among the gram positive isolates, 12.85% were multidrug resistant out of which resistance were mostly seen against ampicillin (70.75%), cotrimoxazole (51.23%) and ciprofloxacin (25.29%) whereas sensitivity of gentamycin, cloxacillin and vancomycin were 85.78%, 88.94%, 100% respectively. Similarly among the gram-negative isolates, 31.5% were multidrug resistant. Higher rate of resistance were seen against ampicillin (88.17%), ceftrianone (74%) cotrimoxazole (63.49%) and ciprofloxacin (53.17%), but were susceptible to gentamycin (75%) and imipenem (94.22%) and tigecyceline (97.94%). Both gram positive and gram negative organisms are equally responsible for causing wound infection. In Conclusion, Gram positive organisms isolated from pyogenic infections comparatively offer a wide range of antimicrobial therapeutic options than gram negative isolates. For gram positive organisms, cloxacillin can be used for initial empirical treatment. Since our study showed a higher rate of resistance in gram negative infections, they usually produce limited therapeutic options. Even though limited data are available, one should always consider the possibility of fungal etiology, especially when the patient is not improving despite administration of antibiotics for a significant duration of time.

KEYWORDS

Pus Culture, Swab Culture, Multi-drug Resistance,

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INTRODUCTION

A whitish yellow or yellow liquid accumulation of dead white blood cells and dead bacteria produced during an inflammatory pyogenic infection is called pus.[1] Accumulation of pus in an enclosed tissue space produces an abscess. Pus can be formed in different anatomical sites by a wide ranges of microbial species which can be endogenous or exogenous organisms, polymicrobial or monomicrobial.[2]

The most common pus producing bacteria are *Staphylococcus aureus, Klebsiella spp.*, *Pseudomonas spp, Escherichia coli, Streptococci spp*.[3,4] Several studies done across the world on wound infections have been consistent enough to show a predictable bacterial profile. This makes for an important observation for a clinician who intends to start empirical treatment for his/her patients while laboratory culture reports are awaited.[5] Selection of an effective antimicrobial agent requires a good knowledge of the involved pathogen, pathophysiology of the infectious process and also an understanding of pharmacology of the intended therapeutic agents .[6]

The reasons for increase in multiple problems like prolonged hospital stay, economic burden and significant morbidity and mortality may be due to increased incidence of drug-resistant microorganisms .[7] In under developed countries like Nepal where clinicians are forced to use broad spectrum antibiotics due to lack of proper culture and susceptibility testing data, these problems have accelerated and the chances of spread of drug resistant bacteria are major threats.

This study was performed with the objective of finding the prevalence of gram positive and gram negative aerobic pathogens responsible for causing wound infections in our setting, their antibiograms, the percentage of multidrug resistance (MDR) organisms.

MATERIALS AND METHODS

This hospital based prospective cross sectional study was done in the Department of Microbiology, NMCTH, Nepal from Feb 2015 to Jan 2016. Pus samples received at the Microbiology laboratory were processed as per standard laboratory procedures on Blood agar, Chocolate agar and MacConkey agar plates. The culture plates were incubated aerobically at 37^0C for 24 hours. The specific identification of bacterial pathogens grown was done based on their colony morphology, gram's staining characteristics, biochemical properties using standard laboratory procedures .[8]

Antibiotic sensitivity testing was performed using modified Kirby Bauer disc diffusion method on Mueller Hinton agar plate. Zone diameters of inhibition around each disc was measured using a calibrated ruler and interpreted according to Clinical and Laboratory Standard Institute (CLSI) guidelines[8]. The antibiotics discs and the concentrations used were ampicillin (10 µg), cefixime (5 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), gentamycin (10 µg), for Enterococcus gentamycin (120 µg), piperacillin / tazobactam (100/10 µg), imipenem (10 µg), vancomycin (30 µg), cloxacillin (5 µg), polymyxin B (300 u), tigecycline (15 µg). cefoxitin (30mcg) disc was used for screening of MRSA. Vancomycin screening agar was used for Methicillin-resistant *Staphylococcus aureus* (MRSA) Isolates. Organism resistance to more than 2 classes of antibiotics was considered as MDR. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC25922 were included as control strains.

RESULTS

Out of 1052 pus and swab samples, 502(47.72%) showed positive results for aerobic culture. Among 502 samples, 249 were gram positive organisms, 242 gram negative organisms, 7 showed a mixed growth pattern(4 gram positive and 8 gram negative) and 4 showed growth of *Candida albicans* (Figure 1). The most common isolate was *Staphylococcus aureus* (41.45%) followed by *Escherichia coli* (22.79%), *Acinetobacter calcoaceticus- Acinetobacter baumannii* complex (ACB complex) (11.00%) and others.

Out of 211 *Staphylococcus aureus*, 21 were MRSA (9.96%). Among the total gram positive bacterial isolates(n=253), 12.85% were multidrug resistant out of which resistance were mostly seen against ampicillin (70.75%), cotrimoxazole (51.23%) and ciprofloxacin(25.29%) whereas sensitivity of gentamycin, cloxacillin and vancomycin were 85.78%, 88.94%, 100% respectively (Table 1).

Out of 252 gram negative isolates, most common organism was *Esc. Coli* (n=116,) followed by ACB complex (n=56) (Picture 1). Among the gram-negative isolates, 31.5% were multidrug resistant. Higher rate of resistance were seen against ampicillin (88.17%), ceftriaxone (74%) cotrimoxazole (63.49%) but were susceptible to tested aminoglycosides (73.4%), carbapenems (93.47%) and tigecycline (97.94%) (Table 2).

Table 1: Antimicrobial resistance patterns of gram-positive organisms

Antibiotics	***CoNS(n=27)***	Enterococcus spp(n=9	S. aureus (n=211)	S.pyogenes(n=6)	Total Resistance % among tested
Ampicillin	13(48.15%)	2(22.23%)	160(76.19)	2(33.34%)	70.75%
Cephalexin	2 (7.41%)	NT	50 (23.86%)	0(0.00%)	21.32%
Cotrimoxazole	10 (37.03%)	NT	113(53.81)	2(33.34%)	51.23%
Ciprofloxacin	6 (22.23%)	1 (11.12%)	57 (27.14%)	0 (0.00%)	25.29%
Gentamicin*	2 (7.41%)	1 (11.12%)	32 (15.24%)	1 (16.67%)	14.22%
Cloxacillin	4 (14.81)	2 (22.23%)	21 (9.96%)	1 (16.67%)	11.06%
Vancomycin	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0.00%

**Gentamycin High Level (120Microgram) for Enterococuus*

Table 2: Antimicrobial resistance patterns of gram-negative organisms

Antibiotics	E.coli (n=116)	ACB complex (n=56)	K. pneumoniae (n=32)	P. aeruginosam (n=14)	C. freundii (n=14)	Enterobacter Species (n=10)	Proteus species (n=10)	Total Resistance%
Ampicillin	98 (84.48%)	52 (92.86%)	NT	NT	14 (100%)	NT	NT	88.17%
Ceftriaxone	90 (77.59%)	43 (76.78%)	25 (78.13%)	8 (57.14%)	9 (64.28%)	8 (80%)	5 (50%)	74%
Cefixime	85(73.2 %)	40 (71.34%)	21 (65.2%)	NT	9 (64.28%)	8 (80%)	7 (70%)	71.42%
Cotrimoxazole	80 (68.97%)	34 (60.71%)	13 (40.63%)	12 (85.71%)	7 (50%)	6 (60%)	8 (80%)	63.49%
Ciprofloxacin	66 (56.90%)	42 (75%)	12 (37.5%)	6 (42.86%)	4 (28.57%)	2 (20%)	2 (20%)	53.17%
Piperacillin/ Tazobactam	45 (36.84%)	52 (92.86%)	16 (50%)	2 (14.28%)	1 (7.14%)	NT	0 (0.00%)	47.94%
Gentamycin	23 (19.83)	31 (55.36%)	6 (18.75%)	3 (21.43%)	2 (14.28%)	1 (10%)	0 (0.00%)	25%
Polymyxin-B	15 (13.18%)	2 (3.57%)	0 (0.00%)	NT	1 (7.14%)	NT	0 (0.00%)	7.89%
Imipenem	6 (5.26%)	8 (14.28%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	NT	0 (0.00%)	5.78%
Tigecycline	0 (0%)	4 (7.14%)	0 (0.00%)	0 (0.00%)	1 (7.14%)	NT	0 (0.00%)	2.06%

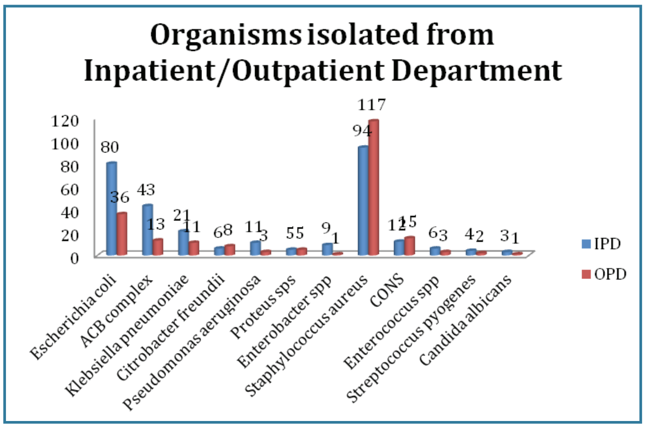

Fig. 1: Organisms isolated from Inpatient/Outpatient Department

DISCUSSION

A positive isolation rate of 47.72% as seen in our study is comparable to that shown by Mulu *et al* while Azene KM *et al* have shown a 70.5% isolation rate.[9,10] The variation in sample type, size, source and laboratory facilities available may account for the variation in isolation rate. The possible delay in sample being transported to the laboratory and the omission of looking for anaerobes may help to explain the lower isolation rate in our study.

Our study has shown that gram positive and gram negative organisms have comparable isolation rates from pus/swab samples; *Staphylococcus aureus* (41.45%) being the predominant one. This finding is similar with the findings of Azene MK *et al* where they have found *Staphylococcus aureus* as the most common organism (41.6%) causing wound infection.[10] Similarly Tiwari P *et al*. has also reported *Staphylococcus aureus* as the most common organism isolated from pus (35.5%).[11] In contrast with these findings, reports from Nigeria have shown *Pseudomonas aeruginosa* as being the most common isolates from pus sample.[12] Such difference in findings could be due to geographical variations or due to the different sources of samples being processed.

The prevalence of MRSA in our study was 9.96% which is similar to 17.5% as found in the study done in Ethiopia.[13] This prevalence rate of MRSA is much lower then the prevalence reported from India (unto 41%).[14] This signifies that the cloxacillin can still be the best empirical antibiotics to treat the community acquired wound infections in our settings. Other gram positive organisms causing wound infection in our study were CoNS (5.30%), *Enterococcus* (1.76%) and *Streptococcus pyogenes* (1.18%). Most of the gram positive organisms were isolated from samples received from out patients and were sensitive to vancomycin (100%), cloxacillin (88.94%), gentamycin (85.78%), ciprofloxacin (74.71%) and resistant to ampicillin (70.75%) followed by cotrimoxazole (51.23%). This pattern of antibiotic susceptibility seen in our study could be attributed to the predominant isolates being obtained from outpatient department thereby reflecting the community at large.

E.coli was the most common among the gram negative isolates accounting for 22.78%. This finding is similar to the finding of Azene MK *et al.*[10] In our study, 11.00% of isolates were ACB complex which is similar to 13.4% as reported in a hospital based study from Nepal.[15] Among gram negative isolates, ampicillin (88.17%) showed a higher degree of resistance followed by ceftriaxone(74%). Similar susceptibility patterns were also observed in a study by Iffat Javed *et al.*[16] Comparatively, aminoglycoside showed lower percentage of resistance (25%). Imipenem (94.22%) and tigecycline (97.94%) where tested were fairly sensitive for those organisms showing resistance to ampicillin, cephalosporins and aminoglycosides as also seen by Balan *et al.*[17] This finding suggests that the higher antibiotics like carbapenem and tigecycline are still very effective in treating gram negative infections.

In our study, most of the gram negative organisms were isolated from inpatients. This may be the reason for the higher incidence of drug resistance noticed among gram negative isolates. Similar type of resistance patterns were noticed among gram negative isolates reported in a WHO community surveillance report on pathogenic organisms, which suggested that more number of resistant organisms were observed among hospitalized patients than in those in the community.[18] The predominance of mono- microbial infections observed in our study is substantiated by a study done by Basu S *et al.*[19] Such observations may be due to the limited laboratory facilities in our setting, starting from the point of sample collection and transport to the end of sample processing where we didn't consider anaerobiasis.

In the present study, 4 (0.79%) swab samples cultured grew *Candida albicans*, all of which were reflectory to higher classes of antibiotics treatment. Hence, occurrence of fungal agents can't be neglected in cases where patients are not improving despite antibiotics treatment. Similar prevalence (0.8%) of fungal aetiology was reported in a study conducted in India by Hanumanthapa P *et al.*[20] Isolation of *Candida species* from different wound sample has been reported by different authors.[21] This supports the fact that pus samples received in the laboratory may need to be processed to seek fungal pathogens and the occurrence of such fungal agents may not be neglected in cases where patients are not improving despite antibiotic treatment.

In conclusion, both gram positive and gram negative organisms are equally responsible for causing wound infection. Gram positive organisms isolated from pyogenic infections comparatively offers a wide range of therapeutic options than gram negative isolates. For gram positive organisms, cloxacillin and quinolones can be used for empirical treatment. Since our study showed higher rates of resistance in gram negative organisms, they usually produce limited therapeutic options. Even though limited data are available, one should always consider the possibility of fungal etiology, especially when the patient is not improving despite administration of antibiotics for significant duration of time.

Declaration of Interests: The authors declare no conflicts of interest.

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