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Bacterial Isolates From Pus and their Antibiogram Patterns Among Patients at Teule Hospital DDH, Muheza

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ABSTRACT

A retrospective study of bacterial isolates from pus and their susceptibility pattern was done among patients at Teule Designated District Hospital, Muheza, Tanzania from October 2015 to August 2016. A total of 156 bacterial isolates were reviewed on the basis of their susceptibility pattern. Of these, 59 (37.82%) were *Staphylococcus aureus* followed by *Proteus mirabilis* 21 (13.46%), Coliforms 19 (12.17%), *Pseudomonas aeruginosa* 15 (9.61%), *Escherichia coli* 12 (7.69%), *Klebsiella pneumoniae* 5 (3.20%), *Acinetobacter* species 3 (1.92%), *Streptococcus pyogenes* 4 (2.56%), *Klebsiella oxytoca* 1 (0.64%) and mixed organisms 17 (10.89%). The sensitivity pattern showed that those sensitive to ciprofloxacin were 128 (92.8%), gentamicin 117 (81.3%) and ceftriaxone 50 (69.4%) while others were resistant to tetracycline 40 (67.6%), clotrimoxazole 82 (64.6%) and erythromycin 39 (57.4%). The study reveals that *S. aureus* is the leading cause in pus isolates. Ciprofloxacin ($p = 0.5$), gentamicin ($p = 0.05$) and ceftazidime ($p = 0.02$) are the most effective and can be used empirically in treating these conditions though only gentamicin and ceftazidime are showing statistical significance. Resistance shown to erythromycin, tetracycline, ampicillin and cotrimoxazole may be due to being used over a much longer period of time and to indiscriminate use by health professionals.

Keywords: Muheza, Designated District Hospital, Antibiogram, Mixed organisms, Coliforms.

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INTRODUCTION

Infection of the wound is the successful invasion and proliferation by one or more species of microorganism anywhere within the sterile tissues in body that results in pus formation. Pus is viscous exudates, typically whitish-yellow, yellow, or yellow or yellow brown, formed at the site of inflammation during infection ¹. Bacteria isolated from pus had been a problem especially when it comes to the surgical site unit in all age groups and those who develop blister following a certain parasitic infection. It is not a categorical problem rather it is a worldwide problem. There is no enough measure undertaken to overcome this problem, especially when it comes to selection of antimicrobials due to resistance shown by most of the bacterial isolates obtained from pus ².

Pyogenic bacteria such as *Staphylococcus species*, *Proteus species*, *Escherichia coli*, *Klebsiella species* and *Pseudomonas aeruginosa* has been a threat for most of the cases in surgical site unit and this is due to postoperative wounds. Sometimes they become life threatening because certain species can be treated before its spread and after a while it becomes resistance again and that is where the problem comes. Wound infections have been a problem in the field of surgery for a long time. Advances in control of infections have not completely eradicated the problem because of development of resistance ². Most of the drugs had been used for a while now and their resistant pattern has never been considered lately. Bacterial species such as *Staphylococcus species* and *Escherichia coli*, *Klebsiella species* tend to resist most of the antimicrobial patterns. Antimicrobial resistance can increase complications and costs associated with procedures and treatment. An infected wound complicates the postoperative course and results in prolonged stay in the hospital and delayed recovery ³. Although many efforts has been made to reduce or eradicate the problem but still persists when it comes to the results of most of pus culture. There is little information on the prevalence of bacterial isolates from pus and their susceptibility pattern. Therefore this study was done to determine the occurrence of bacterial isolates from pus culture and their susceptibility pattern among patients at Teule Designated District Hospital, Tanzania.

MATERIALS AND METHOD

The study design of the present project is an analytical, hospital based retrospective study conducted in Teule hospital. A total of 156 patients were used to analyze the sensitivity and resistance pattern for the common bacterial isolates from pus. The study was conducted at clinical microbiology laboratory at Teule Hospital DDH, Muheza located in northern Tanzania about 4 km from Tanga town. The study covered 2 years, i.e the data which was collected from October 2015 to August 2016. All isolated bacterial culture of pus that has been performed before October 2015 to August 2016 was excluded from this study.

Systematic sampling method was used and the data recorded from the entire patient was used. Sample size was based on the minimum sample size required to have 90% of positive results, 5% level of significance and confidence interval of 95%. The sample size estimated was 156, which was the total number of patients and their sample results from pus culture.

The sample size was calculated using the formula:

$$n = Z^2 p(1-p) / \epsilon^2$$

Where n=maximum sample size: z = Standard normal deviation: Proportional from literature review: ϵ = Maximum likely error tolerance

Method:

Patient's microbiological records were reviewed from October 2015 to August 2016 and patient's age, gender, bacterial isolates and susceptibility pattern was recorded in the checklist.

Data collection tool:

All the data was collected from the patients' results from the Clinical Microbiology Laboratory at Teule Designated District Hospital, Muheza, Tanzania. The data was collected using the checklist tool, where patient's record and findings were obtained and recorded in the specified time.

Data analysis:

Analysis of data was carried out using Statistical Package for Social Sciences (SPSS) version 16.0. Categorical data was analysed by using Pearson Chi-square (χ^2) test. Standard deviation (S.D) and standard error (SE) was used to describe the spread around the mean of the sample. A p-value of 0.05 was considered the measurer of statistical significance.

Ethical clearance:

Permission to conduct the study was sought from Designated District Hospital -Ethical clearance committee. Permission to review patient's records was sought from Head of Designated District Hospital. Confidentiality was adhered to whereby data collection sheet was containing patient's identity number instead of patient's name. The patient's information was only available to the researcher.

RESULTS AND DISCUSSION

The results are based on the study population, patient age in years, age category of the patients, bacteria isolated and sensitivity testing among the isolated organisms for the drugs selected is given below.

Population characteristic:

A total of 156 patients were selected in the study. Of this, 61 were females (39%). The mean age was (Mean \pm SD) 24.4 \pm 20.3. The population characteristics of the patients are shown in Table 1.

Table 1: Characteristic of age, gender, mean and standard deviation among study participants

Variable	Measure
Mean \pm SD	24.4 \pm 20.3
Gender, Female % (n/N)	39%(61)

Age category among patients:

Patients were categorized according to their age. The patients falling in the age group between 18 years and above were 93 (59.6%), between 6 years and 17 years were 20 (12.8%) and between 5 years and below were 43 (27.5%). The results are presented in Table 2.

Table 2: Age category of patients and the frequency of each in their category

Age in year's	Frequency	Percentage (%)
\leq To 5	43	27.6
6 to 17	20	12.8
\geq 18	93	59.6
Total	156	100

Bacterial isolates:

Results (Table 3) show that out of 156 samples, nine different bacteria were isolated. In other samples mixed organisms were isolated. The mixed organisms were treated as a single group. *S. aureus* was the most common bacteria to be isolated in 59 of the isolates (37.82%), followed by *P. mirabilis* 21 (13.46%), Coliforms 19 (12.17%), *P. aeruginosa* 15 (9.61%), *E. coli* 12 (7.69%), *K. pneumonia* 5 (3.20%), *Acinetobacter sps* 3 (1.92%), *S. pyogenes* 4 (2.56%), *Klebsiella oxytoca* 1 (0.64%) and the mixed organisms were 17 (10.89%).

Table 3: Bacterial isolates from the pus

Name of the Bacterial isolate	Number of isolated organisms (%)	Percentage (%)
<i>Staphylococcus aureus</i>	59	37.82
<i>Proteus mirabilis</i>	21	13.46
Coliforms	19	12.17
<i>P. aeruginosa</i>	15	9.61
<i>E. coli</i>	12	7.69
<i>K. pneumonia</i>	5	3.20
<i>S. pyogenic</i>	4	2.56
<i>Acinetobacter</i> species	3	1.92
<i>K. oxytoca</i>	1	0.64
Mixed organisms	17	10.89
Total	156	100

Antibiogram of isolated organisms:

The results of antibiotic sensitivity of all the isolated bacterial species are shown in Tables 4a-4c.

Antibiotic sensitivity result shows that most isolates were sensitive to ciprofloxacin; *K. pneumoniae* 100% (4), *S. pyogenes* 100% (4), *P. mirabilis* 93.8% (15) and *P. aeruginosa* 93.3% (14) respectively. The chi-square (χ^2) = 15.2 and P-value 0.5, are not statistically significant because the number of sensitive organisms was not quiet related to the isolated organisms.

S. pyogenes isolates were 75% (3) sensitive to erythromycin. The chi-square (χ^2) was 6.4 and the P-value was 0.6 which was not statistically significant because the isolated organisms were not same in the sensitive results due to few sample size obtained.

Gentamicin was sensitive to most of the organisms; *S. pyogen* was 100% (4), *E. coli* 90.9% (10), *P. mirabilis* 90% (18) and *S. aureus* 88.9% (48) sensitive. *K. pneumoniae* 60% (3) and *Acinetobacter* species 66.7% (2) were resistant to gentamicin respectively. The chi-square (χ^2) was 25.8 and the P-value was 0.05 which was statistically significant.

Tetracycline was resistant to most of the isolates tested; *E. coli* was 83.3% (5), *P. mirabilis* 75% (12) and Coliforms 67.7% (10) were resistant. *P. aeruginosa* showed less resistance in that only 50% (2) were resistant to tetracycline. The chi square (χ^2) was 6.0 and the P-value was 0.7 which was not statistically significant.

Ceftriaxone was sensitive when tested against *S. pyogenes* 100% (3) and Coliforms 100% (2), *P. mirabilis* 80% (8). *P. aeruginosa* 66.7% (8) and *K. pneumoniae* 66.7 % (2) resistant against ceftriaxone. The chi square (χ^2) was 24.4 and P-value was 0.05 which was statistically significant.

Another antibiotic was ceftazidime which was 93.8 % (15) sensitive with Coliforms and 92.3% (12) with *P. mirabilis*. *S. aureus* was 73.1 % (19) sensitive. *Acinetobacter* species and *K. oxytica* were 100 % (2) resistant when tested with ceftazidime. The chi square (χ^2) was 19.4 and the P-value was 0.02 which was statistically significant.

Table 4(a): Antibiotic susceptibility to bacterial isolates

Antibiotics Bacterial Isolates	Ciprofloxacin		Erythromycin		Amikacin	
	S	R	S	R	S	R
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<i>Staphylococcus aureus</i>	49(90.7)	5(9.3)	14(40)	21(60)	11(68.8)	5(31.8)
Coliforms	13(92.9)	1(7.1)	4(36.4)	7(63.7)	2(40)	3(60)
<i>Proteus mirabilis</i>	15(93.8)	1(6.3)	3(37.5)	5(62.5)	2(33.3)	4(66.7)
<i>P. aeruginosa</i>	14(93.3)	1(6.7)	-	-	10(90.9)	1(9.1)
<i>K. pneumoniae</i>	4(100)	0 (0)	0 (0)	2(100)	1(100)	0 (0)
<i>E. coli</i>	11(91.7)	1(8.3)	2(50)	2(50)	0 (0)	2(100)
<i>Acinetobacter</i> species	3(100)	0 (0)	-	-	2(67.7)	1(33.3)

<i>S. pyogenic</i>	4(100)	0 (0)	3(75)	1(25)	-	-
<i>K. oxytoca</i>	1(100)	0 (0)	-	-	1(100)	0 (0)
Mixed organisms	14(93.3)	1(6.7)	3(75)	1(25)	6(85.7)	1(14.3)
p- value	= 0.5		= 0.6		= 0.08	

R= Resistant; **S** =Sensitive; n= number of isolates; **dash(-)** = organisms not tested for particular antibiotic.

Table 4(b): Antibiotic susceptibility to bacterial isolates

Antibiotics Bacterial Isolates	Tetracycline		Gentamicin		Cotrimoxazole	
	S	R	S	R	S	R
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<i>Staphylococcus aureus</i>	8(42.1)	11(57.9)	48(88.9)	6(11.1)	19(38.8)	30(61.2)
Coliforms	5(33.3)	10(67.7)	12(66.7)	6(33.3)	5(26.3)	14(73.7)
<i>Proteus mirabilis</i>	4(25)	12(75)	18(90)	2(10)	8(44.4)	10(55.6)
<i>P. aeruginosa</i>	2(50)	2(50)	8(57.1)	6(42.9)	4(57.1)	3(42.9)
<i>K. pneumoniae</i>	1(25)	3(75)	2(40)	3(60)	1(20)	4(80)
<i>E. coli</i>	1(16.7)	5(83.3)	10(90.9)	1(9.1)	3(27.3)	8(72.7)
<i>Acinetobacter</i> species	-	-	1(33.3)	2(66.7)	0 (0)	1(100)
<i>S. pyogenic</i>	-	-	4(100)	0 (0)	0 (0)	4(100)
<i>K. oxytoca</i>	-	-	1(100)	0 (0)	-	-
Mixed organisms	1(25)	3(75)	14(93.3)	1(6.7)	5(38.5)	8(61.5)
p- value	= 0.7		= 0.05		= 0.4	

R= Resistant; **S** =Sensitive; n= number of isolates; **dash(-)** = organisms not tested for particular antibiotic.

Table 4(c): Antibiotic susceptibility to bacterial isolates

Antibiotics Bacterial Isolates	Ampicillin		Ceftriaxone		Ceftazidime	
	S	R	S	R	S	R
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<i>Staphylococcus aureus</i>	1(14.3)	6(85.7)	17(77.3)	5(22.7)	19(73.1)	7(26.9)
Coliforms	-	-	2(100)	0 (0)	15(93.8)	1(6.3)
<i>Proteus mirabilis</i>	0 (0)	1(100)	4(80)	1(20)	12(92.3)	1(7.7)
<i>P. aeruginosa</i>	0 (0)	1(100)	4(33.3)	8(66.7)	6(54.5)	5(45.5)
<i>K. pneumoniae</i>	0 (0)	1(100)	1(33.3)	2(66.7)	1(50)	1(50)
<i>E. coli</i>	0 (0)	12(100)	8(80.0)	2(20.0)	1(50)	1(50)
<i>Acinetobacter</i> species	-	-	1(50)	1(50)	0 (0)	2(100)
<i>S. pyogenic</i>	-	-	3(100)	0 (0)	-	-
<i>K. oxytoca</i>	-	-	1(100)	0 (0)	0 (0)	2(100)
Mixed organisms	5(45.4)	6(45.6)	9(75)	3(25)	4(100)	0 (0)
p- value	= 0.09		= 0.05		= 0.02	

R= Resistant; **S** =Sensitive; n= number of isolates; **dash(-)** = organisms not tested for particular antibiotic.

DISCUSSION:

The knowledge of bacterial infection and laboratory susceptibility testing of the isolated organisms could make a rational selection for the drugs for treatment ⁴. Prolonged infection on the skin, eye, ear or wound is common that there will be accumulation of pus ⁵. Patients from surgical sites had been affected with prolonged wound infection which in time results in

trauma care, prolonged hospital stay and treatment ⁶. Moreover hospitals are facing crisis over the increase and dissemination of antimicrobial resistant bacteria, particularly those caused by abscess from different wound patients ⁷.

In this study the results shows that *S. aureus* is the leading etiological agent to be isolated as it accounts for 37.8% (59) followed by *P. mirabilis* 13.5% (21). Both of these two species are good causative agents of pus formation in postoperative wounds, brain abscess and burn wounds. Other organisms which also were associated with infection were Coliforms, *P. aeruginosa*, *E. coli* and *K. pneumoniae*. This is similar to the study conducted by Anguzu and Olila *et al* in 2007 about drug sensitivity pattern on septic-post operative wounds in Uganda that also showed that the leading etiological agents from pus culture were *S. aureus*, Coliforms, *P. mirabilis*, *P. aeruginosa* and *K. pneumoniae* ⁸. Pus production is a common manifestation of infection due to *S. aureus* in tissues and sites with lowered host resistance such as damaged skin and mucus membrane, where it may produce skin lesions such as boil or surgical site infection ⁸.

The occurrence of *S. aureus* infection may be because it is an endogenous source of infection. Nasal carriage of *S. aureus* is an important risk factor for infection of surgical site as the organism is a normal flora in the nostrils. Infection with this organism may also be due to contamination from the environment e.g. contamination of surgical instruments with the disruption of natural skin barrier. *S. aureus*, which is a common bacterium on surfaces, easily find their way into surgical sites ⁹.

The susceptibility testing shows that the bacterial isolated were highly susceptible to ciprofloxacin with a certain variance to gentamicin with a chi-square(χ^2) of 15.2 and 25.8 respectively while their P-values were 0.5 and 0.05 respectively where by gentamicin was statistical significant while ciprofloxacin was not. Resistance was seen in some of the commonly used drugs such as tetracycline, clotrimazole and ampicilin. From the results the preferred antibiotics for the therapy following the infection of either of the isolated organisms are clotrimoxazole, gentamicin, erythromycin, tetracycline and ampicilin. And the most probable reasons for their selection are that these antibiotics had been in the market for long, relatively cheap and readily available ¹⁰.

However, resistance among the selected antibiotics as erythromycin was 60% (21) while only 14.3 % (1) of *S. aureus* was sensitive to ampicilin. The resistance shown by erythromycin, tetracycline, ampicilin and clotrimoxazole may be due to the antibiotics having been in use for much longer time and their oral route of administration that affects their rate of absorption into blood stream. Some of them were used as prophylaxis therefore increasing their use in patients. Over-use of antibiotics contributes to organisms developing resistance ¹¹. The

emergence of resistance to antimicrobial agent is a global public health problem especially among pathogen causing infection. This is essential due to improper use of antibiotic by health professionals, unskilled practitioners and lay persons, poor drug quality and inadequate surveillance program ¹².

Ceftriaxone and ceftazidime are third-generation cephalosporins that are relatively rare in the hospitals and are expensive. Their high cost and being less readily available to patient's means these drugs have not been misused and hence are more effective compared to those that have been in use for quite a long time.

In this study, it has been observed that, *S. aureus* is the leading etiological agent in pus producing infections and that ciprofloxacin and gentamicin may be used in the treatment of such infection before microbial and sensitivity test are carried out. In turn it was resistant towards erythromycin 60% (21), tetracycline 57.9% (11) and ampicilin 85.7 % (6). This is similar to the study conducted by Nizamiet *al.*, in 2012 that from the sample collected from pus the strains of *S. aureus* were resistant to tetracycline and erythromycin ¹³. Gentamicin was 90% (14) sensitive while only 25% (4) of *P. mirabilis* was sensitive to tetracycline. Also *S. pyogenes* was 100 % (4) sensitive to gentamicin and ciprofloxacin the same percent was resistant towards clotrimoxazole for the isolated organisms. The presence of Coliforms (14.3%), *P. mirabilis* (14.3%), *E. coli* (10.7%) and *Enterobacter* species (7.1%) can be due to contamination of wounds with patient's endogenous flora. *E. coli* and coliforms is normal flora of gastro-intestinal tract ⁹.

High level of resistance to common antibiotics encountered in most of the isolates of bacterial pathogens is an indication that control measure has to be put in place, particularly in the administration of antibiotics in hospitals. Patients should be educated on the consequence of indiscriminate use of antibiotics and their consequences and there should also be resistance antibiotic surveillance scheme. Adequate epidemiological data on characterization and antibiotic susceptibility of bacterial pathogen is an essential ingredient to guide empiric antibiotic therapy in the ambulatory setting. This will reduce or eliminate errors in empirical selection of either infective or expensive drugs, prolonged hospitalization and higher mortality.

CONCLUSION

In view of the fact that a certain number of isolated organisms are resistant while others are sensitive towards the tested antibiotics, most sensitive antibiotics, ciprofloxacin and gentamicin, should be used when treating against organisms isolated from pus specimens. The use of antibiotics such as tetracycline and ampicilin showing high level resistance to

organisms such as *S. aureus* and *P. aeruginosa* respectively should be reconsidered. Based on the findings of the present study the following recommendations are made:-

Empirical treatment for the bacterial isolated from pus provokes drug resistance. Therefore, the treatment should be based on the results of sensitivity pattern.

Antibiotics as ciprofloxacin, ceftazidime and gentamicin could be used for the treatment of bacterial isolated from pus from different sites based on antibiotic susceptibility testing. There is a need for continuous surveillance for resistant bacteria to provide the rationale for alternative treatment

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