Original Research Article

Evaluation of Clinico-epidemiology profile and susceptibility pattern of bacterial isolates of purulent samples in a university hospital, India

Abstract

Background: Patients suffering from trauma, burns or those who have undergone surgical interventions are susceptible to pyogenic infections caused by commensal as well as exogenously acquired pathogens. This study helps us recognize and characterize aerobic bacteria isolated from pyogenic samples and establish antibiotic resistance for clinicians to devise an empirical treatment for patients suffering from similar disease.

Methods: This observational study was carried out from November 2019 to May 2020 in the Department of Microbiology at a teaching hospital in northern India and included all patients who presented with suppurative infections during the study period.

Results: Total 201 culture positive samples were included in our study of which 80 (40%) samples were of sterile body fluids, 51 (25%) samples were of pus aspirates and the other samples included wound pus and surgical site infection samples. *Escherichia coli* was the most common pathogen (30.34%) followed by *Klebsiella pneumoniae* (17.91%) and *Pseudomonas spp* (13.43%).Sixty eight (33.83%) isolates were multidrug resistant. *Enterobacter spp* was completely resistant to ciprofloxacin, ceftazidime and ceftriaxone, and 25% sensitive to Cefoperazone-Sulbactam, thus showing the presence of extended spectrum beta lactamase (ESBL) character. We studied the association of multidrug resistance to certain risk factors like previous antibiotic therapy, days of wound before admission, increased length of hospital stay,

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patient comorbidities like diabetes mellitus, heart disease, chronic liver disease and immunosupression.

Conclusions: This study reveals Gram negative bacilli to be more commonly causing pyogenic infections in our patients. Local antibiotic susceptibility profile of these pathogens is essential for empirical treatment.

Keywords: Purulent; Bacteria; Antibiotic Resistance; Clinical; Multi Drug Resistance

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Introduction

Patients suffering from trauma, burns or those who have undergone surgical interventions are susceptible to pyogenic infections caused by commensal as well as exogenously acquired pathogens [1]. Bacteria proliferate better in the presence of nutrition, warmth, moisture in wound and they propagate from either from the environment or from the patients' commensal flora and demonstrate virulence factors producing wound infection [2]. Wound infections caused by aerobic and anaerobic bacteria have been known to be acquired nosocomially, resulting in amplified morbidity, increased days of hospital stay leading to increased expenditure [3]. The rapid spread of antibiotic resistance among pathogenic bacteria is considered as serious threats to the health of people worldwide. Inadequate dose regimen of antibiotics and misinterpretation of the antibiotic sensitivity test report has lead to the emergence and reemergence of multidrug resistant *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *methicillin-resistant Staphylococcus aureus* (*MRSA*) related to nosocomially acquired pyogenic disease [4-6]. Among many studies conducted worldwide, the bacteria causing wound infection is almost similar with variation in the antibiotic sensitivity among diverse geographical area. Inappropriate use of antibiotics and not observing strict hospital

infection control measures have lead to surfacing of drug resistant pathogen that lead to increased length of hospitalization and complications along with increased cost of treatment [7]. With increase in length of hospital stay the patient infected with the multidrug resistant bacteria is capable of transferring it to other patients, relatives or healthcare workers. So, we should be well versed with the microbial profile and antibiotic sensitivity of bacteria commonly isolated from wound infection for providing best treatment of patients. The intension of this study is to unearth the microbiological characteristics and drug resistance patterns of bacterial pathogens isolated from the pus exudates at our hospital to guide clinicians in formulating a proper antibiotic regimen for treatment of the patients prone to suffering similar infections.

Aims and Objectives

1. Identification and characterization of the microorganisms isolated from pyogenic samples.

2. To ascertain the antibiotic resistance among the microorganisms isolated.

Materials and Methods:

This study was carried out retrospectively in Bacteriology section of the Department of Microbiology at a teaching hospital in Northern India from November 2019 to May 2020 for a period of Six months.. The study was approved by institute ethics committee (IEC-IMP-2021-188). Our patient group included those who visited the outpatient departments and also those who were admitted in the Inpatient departments of the institute with suppurative infections were the study population from whom the pus samples were collected. The pus samples obtained in our laboratory prominently consisted of pus exudate from furuncles, pustules and abrasions and

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the other samples that were sent to our laboratory were pus exudates from nasal wounds, ear discharges, wounds or abscesses from legs, surgical site infections and sterile body fluids. Initially a Grams stain was done for all the samples and Blood agar and Mac Conkey agar plates were inoculated and further incubated at 37°C for 48 h. staining by Grams' method, microscopic features, characteristic of the colony obtained on culture plates, and biochemical tests were used to identify and characterize the bacterial isolates [8]. Antibiotic discs containing Amikacin (30mcg), Ampicillin (10mcg), Ampicillin-Sulbactum (10/10mcg), Azetreonam (30mcg), Ceftazidime (30mcg), Ceftriaxzone (30mcg),Cefoperazone-Sulbactum (75/10mcg), Ciprofloxacin(5mcg), Cefoxitin (30mcg), Clindamycin (2mcg), Doxycycline(10mcg), Ertapenem (10mcg), Gentamicin (10mcg), Erythromycin (15mcg), Imipenem(10mcg), Levofloxacin (5mcg), Linezolid (30mcg), Meropenem (30mcg), Minocycline (30mcg), Tigecycline (15mcg), Trimethoprim-sulphamethoxazole (1.25/23.75mcg), Teicoplanin (30 mcg), Piperacillin-tazobactam (100/10mcg), Vancomycin (30mcg) and Colistin (0.016-256mcg) Epsilometeric test strips were procured from the company bioMéreux and were used according to the instructions of the manufacturer. Kirby Bauer disc diffusion method was used to perform the antibiotic sensitivity testing using Epsilometeric test strip and disc diffusion test, in compliance with the norms of the Clinical and Laboratory Standards Institute [9]. While performing the antibiotic susceptibility testing, inoculums with a turbidity of 0.5 McFarland were prepared for the bacterial isolate and it was lawn cultured on Muller-Hinton agar plates, following which the antibiotic discs and E-strips were placed on the inoculated plates and incubated at 37º C overnight. CLSI guidelines were used to classify the antibiotics as sensitive, intermediate, and resistant [9]. Statistical analysis were performed using the software program

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IBM SPSS Statistics version 20.0 (SPSS Inc.), with $p < 0.05$ which was considered statistically significant.

Result

Demographics

A total number of 201 culture positive samples including wound and surgical site infections, sterile body fluids and pus samples were collected for 6 months and included in our study. Among these culture positive patients 80 (39.80 %) of them were in the range of 41 to 60 years, followed by 61-70 years and 31- 40 years which were 36 and 32 cases respectively. In our study, samples from male patients were more predominant than samples from women, 104 (51.74%) patients were men and 97 (48.25%) patients were women respectively. In all, the patients who were aged between 41 to 60 years, irrespective of gender are more commonly include in our study (Table 1). Out of the 201 culture positive samples, 80 (40%) samples were of sterile body fluids, 51 (25%) samples were of pus aspirates and the other samples include wound pus and surgical site infection samples (Fig. 1). *Escherichia coli* were the most common isolated bacteria (30.34%) which was followed by *Klebsiella pneumoniae* (17.91%) and *Pseudomonas spp* (13.43%).

Multidrug resistance was commonly encountered when reporting antibiotic sensitivity testing of the isolates included in our study. Sixty eight (33.83%) isolates in our study were Multidrug resistant. The most common comorbidities encountered among the multidrug resistant isolates were malignancy (39.70%), diabetes mellitus (36.76%) and liver disease (36.76%), although they were not statistically significant to the acquisition of multidrug resistance (Table 1). The average

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days of wound before presenting to the tertiary care centre is 4.09 ± 5.547 days. The average length of hospitalization in the patients from our study is 15.35 ± 6.97 days and the positive pus exudate cultures where obtained within an average of 6.19 ± 3.39 days.

Microbiological Characteristics

In patients with purulent exudate samples, *Escherichia coli* (30.3%) was the mostly commonly isolated bacterium, followed by *Klebsiella pneumoniae* (17.9%) and *Pseudomonas spp* (13.4%) (Fig.2). *Escherichia coli* was more commonly isolated in 55.73% of cultures that were positive in community-acquired infection, in comparison with 47.69% for *Escherichia coli* and *Enterococcus faecalis* isolated from pyogenic samples in nosocomially-acquired infections. During hospitalization, the patients developed an infection with *Pseudomonas spp* and *Pseudomonas aeruginosa* in 55.55%, in comparison with 44.44% in community-acquired infection. Among the gram positive microorganism, *Methicillin Resistant Staphylococcus aureus* was isolated in 92.85% from community-acquired infection and *Enterococcus spp* was isolated in 71.42% and 28.57% from community-acquired infection and nosocomial infection, respectively.

Multidrug Resistance

Sixty eight (33.83%) isolates in our study were Multidrug resistant. Out of the most commonly isolated gram negative bacteria, *Enterobacter spp* seems to be the most resistant to most of the first line drugs for lactose fermenting gram negative bacteria; it is completely resistant to Ciprofloxacin, Ceftazidime and Ceftriaxzone, and 25% sensitive to Cefoperazone- Sulbactum, thus showing the presence of Extended spectrum beta lactamase (ESBL) character (Table 2) .

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The *Pseudomonas spp* isolates obtained from our study are most susceptible to Imipenem and Meropenem, with 51.85% susceptibility to each of these drugs, followed by Amikacin, to which the microorganism was 48.14% sensitive. The microorganism was most resistant to Minocycline followed by Ciprofloxacin, with a susceptibility of 0% and 18.51% respectively (Table 2).

The Gram positive cocci isolated from the samples in our study were grouped into *Coagulase negative Staphylococcus*, *Coagulase positive Staphylococcus* and *Enterococcus spp*. Of all the gram positive microorganisms isolated from these samples *Coagulase positive* and *coagulase negative Staphylococcus* and *Enterococcus* isolates were most sensitive to Doxycycline, with 81.48%, 90.90% and 68.18%, respectively, followed by Amikacin among the *Coagulase positive Staphylococcus* and *Coagulase negative Staphylococcus*, with a susceptibility of 81.48% and 63.63% respectively. Whereas all the isolates of *Enterococcus spp* were sensitive to Linezolid and Minocycline, thus proving that none of the *Enterococci* isolates were multidrug resistant (Table 3). The risk factors associated with the acquisition of multidrug resistance are previous antibiotic therapy, days of wound before admission, increased in the length of hospital stay, patients suffering from comorbid conditions like diabetes mellitus, chronic liver disease, heart disease and other conditions causing immunosuppression (Table 1), although none of them were statistically significant in our study.

Discussion

Pyogenic or purulent infections are some of the common reasons for disability and increased length of hospital stay. Morbidity, mortality and even death due to sepsis can result due to severe wound infections, which occurs more commonly in case of multiple drug resistant microorganism. Our study is an effort to identify the causative pathogens, their antibiotic

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sensitivity and comorbidities related to the acquisition of multidrug resistant pathogens in pus samples obtained from various departments at a tertiary care centre of Northern India. In our study it was observed that men (51.74%) were more prone to acquire pyogenic infections in comparison to women (48.45%). Similar male predominance for developing pyogenic infections was observed in studies by Khanam et al [10] and Khan et al [11] where it was reported to be 56.1% and 56.6% respectively. In our study Gram-negative bacteria were the prevailing isolates 68.15% from pus samples compared to Gram-positive bacteria 31.84%, which is similar to a study by Trojan et al [12]. When it comes to the microbial profile of the causative pathogens in the pus samples, the results of our study agree with those of Trojan et al [12] and Zhang et al [13] also agreed upon predominant isolation of *E. coli, K. pneumoniae, and Pseudomonas species* in pus samples. Antibiotic susceptibility testing suggested that the gram negative bacterial isolates were more commonly multi-drug resistant and among them *Enterobacter species* was most resistant to most of the empirically administered drugs. Among gram positive cocci, 51.86% isolates of *Staphylococcus aureus* were Cefoxitin resistant (MRSA) and only 22.23% isolates were resistant to Vancomycin. The *coagulase negative Staphylococcus* isolated in our study also showed 100% sensitivity to Vancomycin and Teicoplanin which correlated with Trojan et al [12]. *Escherichia coli* (30.34%) was the most predominant causative pathogen in pus samples followed by *Klebsiella pneumoniae* (17.91%) which was in concordance with the studies performed by Trojan et al [12] and Zhang et al [13]. In our study we observed *Pseudomonas species* to be the third most common causative pathogen in 13.43% of all the microbial isolates identified. *Escherichia coli* was the most common isolate identified in our study and was conspicuously found to be resistant to Ciprofloxacin (93.44%) and Ceftriaxzone (91.81%). Following *Escherichia coli ,Klebsiella pneumoniae* was the next commonly isolated

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bacterium and was 11.11% susceptible to Imipenem, 19.44% to Amikacin and 5.55% to Ceftazidime, 5.55% to Ciprofloxacin, 96.87% sensitive to Colistin, 28.125% sensitive to Tigecycline. Similar results were also shown by another study by Trojan et al [12]. *Pseudomonas aeruginosa* is 51.85% susceptible to Imipenem, and 48.14% to Amikacin, 22.22% susceptibility to Piperacillin-tazobactam, 25.92% to Ceftazidime, 18.51% to Ciprofloxacin but 100% susceptibility to Colistin and 29.62% susceptibility to Cefoperazone Sulbactum. *Staphylococcus aureus* was the most frequently isolated microorganism by Subha et al [14] and Khanam et al [10] where as in our study *Escherichia coli* was the predominant organism.

These MDR strains isolated in our study accounts for 33.83% of the organisms obtained and were found to be susceptible mostly to the drugs of last resort like Polymyxin (Colistin). The emergence of multidrug resistant microorganisms has led to increased morbidity and mortality. Among the Gram negative microorganisms in our study, *Enterobacter spp* is resistant to all Cephalosporins and only 50% sensitive to second line drugs like Colistin and Minocycline. *Staphylococcus aureus* is 77.77% sensitive to vancomycin, 22.22% to Gentamicin, 11.11% to Levofloxacin, 33.33% to Erythromycin and 81.48% to Doxycycline. *Enterococcus species* showed 100% sensitivity to both linezolid and vancomycin. The geographical areas and climatic conditions exhibit a variable range of multidrug resistance among the bacteria isolated from pus samples. Presence of high drug resistance to many antibiotics is observed in our study among *Pseudomonas aeruginosa, E. coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Enterobacter spp* and emergence of such resistance pattern points towards in compliance to treatment in patients, inappropriate regimens of drugs, inadequate dose adjustment, lack of regional antibiotic sensitivity pattern and the clinicians' confined knowledge about antibiotic sensitivity testing and drug resistance. The multidrug resistant organisms tend to infect patients

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with associated risk factors like age, gender, antibiotic therapy administered previously, number of days of wound before admission, increased length of hospital stay, patient comorbidities like diabetes mellitus, heart disease, chronic liver disease and immunosuppression, although in our study, the association of the risk factors to acquisition of multidrug resistant microorganisms is not statistically significant.

Development of multidrug resistance among microorganisms through the years may be due to attainment of the resistant genes among the organisms. Majority of the patients admitted to our tertiary care center had been undergoing treatment from local hospitals where most of the patients suffered from community acquired or nosocomially acquired multidrug resistant infections that were being treated with second or third line antibiotics, without proper antibiotic susceptibility testing leading to acquisition of multidrug resistance. Hospital infection control team should monitor the hand hygiene practices and environmental cleaning to reduce probability of *Staphylococcal* and *Pseudomonal* infection in wound. Appropriate timing of antibiotic susceptibility testing before starting the empirical treatment, adherence to the drugs sensitive to the particular isolate and reinforcing better infection control starting from smaller hospitals to tertiary care centres would curb the spread of infection by multidrug resistant pathogens.

CONCLUSION

Our study reveals gram negative bacilli to be more common organisms isolated from pyogenic infections in our patients. Local antibiotic susceptibility profile of these pathogens is essential for empirical treatment. Hospital infection control measures should ensure strict compliance to hand

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hygiene practices among the health care providers and other infection control measures to decreased spread of multidrug resistant microorganisms.

Consent for publication

All individuals have given consent to participate in the study.

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Table 1. Clinical factors for isolation Multidrug resistant microorganisms in patients with purulent infections (N=68)

Risk factors and demographic characteristics	Incidence of MDR microorganisms (n=68/201, 33.83%)	p-value	95% CI
Source of infection, %			
Community-acquired	37 (54.41%)	0.566	41.88- 66.55
Nosocomially-acquired	31 (45.59%)	0.566	33.45- 58.12
Cause of purulent infection			
Wound	13 (19.11%)	0.497	10.59- 30.47
Surgical site infection	8 (11.76%)	0.724	5.22- 21.87
Others	47 (69.11%)	0.401	56.74- 79.76
Demographics			
Age, years, mean (SD)	47.56 ± 16.03	0.522	43.61- 51.47
Gender, male/female,%	45.6 / 54.4	0.212	33.45-58.12
Comorbidities			
Diabetes mellitus, %	25 (36.76%)	0.827	25.39- 49.33
Liver Disease, %	25 (36.76%)	0.126	25.39- 49.33
Chronic kidney disease, %	18 (26.47%)	0.350	16.50- 38.57
Malignancy, %	27 (39.70%)	0.353	28.03-52.30
Steroid therapy, %	5 (7.35%)	0.687	2.43-16.33
Cytotoxic drugs, %	27 (39.70%)	0.299	28.03-52.30
Smoking, %	19 (27.94%)	0.925	17.73- 40.15
History			
Days of wound before admission, mean (SD)	4.09 ± 5.547	0.352	2.82- 5.40
Hospitalization period, days, mean (SD)	15.35 ± 6.97	0.343	13.73- 17.00
Days within which culture came positive, mean (SD)	6.19 ± 3.39	0.116	5.39- 7.00

*** p-value <0.05 is significant**

SAMPLES

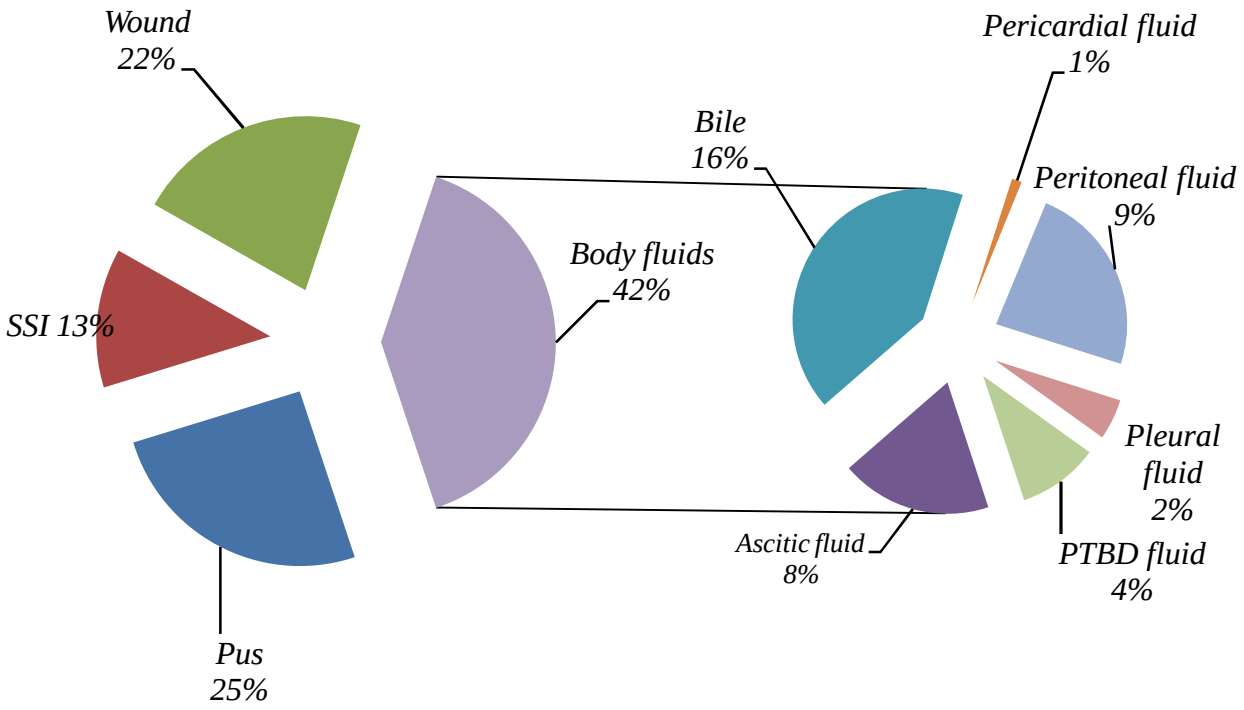

Fig 1. Distribution of types of pus aspirate samples received in our laboratory (N=201)

***SSI** = Surgical site infection

***PTBD** = percutaneous transhepatic biliary decompression fluid

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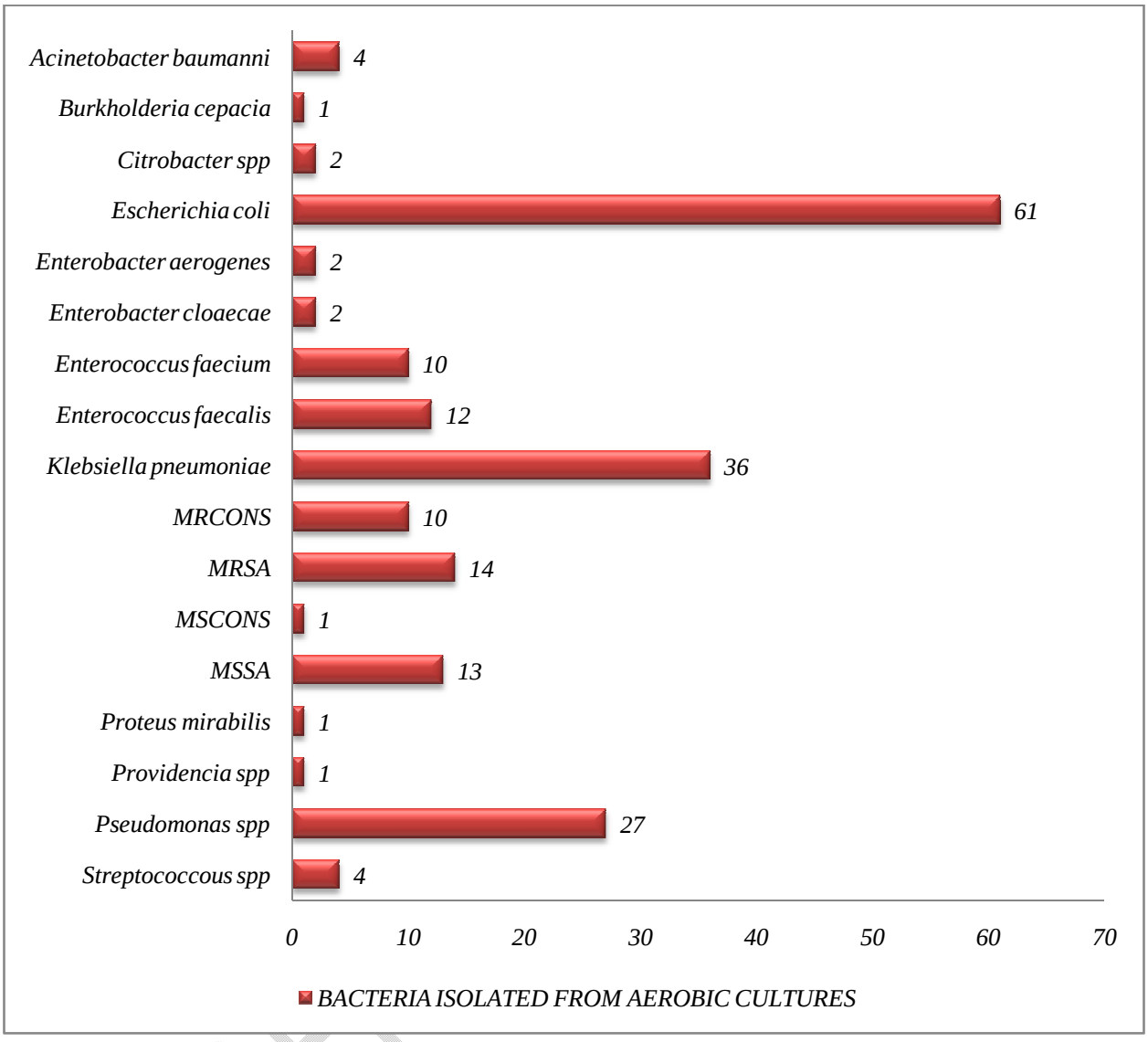

Fig 2. Rate of bacteria isolated from the aerobic pus cultures performed in the laboratory (N=201)

MSCONS- Methicillin sensitive coagulase negative Staphylococcus

MRCONS- Methicillin resistant coagulase negative Staphylococcus

MSSA- Methicillin sensitive Staphylococcus aureus

MRSA- Methicillin resistant Staphylococcus aureus

Table 2: Percentage Sensitivity pattern for first line drugs in most commonly isolated Gram negative bacilli

Antibiotics	E. coli % sensitivity	Enterobacter % sensitivity	K. pneumoniae % sensitivity	Pseudomonas species % sensitivity
Amikacin	81.96%	25%	19.44%	48.14%
Ceftazidime	13.20%	0%	5.55%	25.92%
Ceftriaxzone	8.19%	0%	2.78%	-
Ciprofloxacin	6.56%	0%	5.55%	18.51%
Cefoperazone-Sulbactam	34.42%	25%	5.55%	29.62%
Imipenem	72.13%	0%	11.11%	51.85%
Meropenem	73.77%	50%	11.11%	51.85%
Ertapenem	57.37%	25%	11.11%	-
Colistin	100%	50%	96.87%	100%
Tigecycline	81.25%	0%	28.125%	-
Minocycline	53.33%	50%	53.125%	0%
Azetreonam	-	-	-	14.81%
Piperacillin-Tazobactam	-	-	-	22.22%

Table 3. Percentage Sensitivity pattern for first line drugs in most commonly isolated Gram positive cocci

Antibiotics	Coagulase positive Staphylococcus % sensitivity	Coagulase negative Staphylococcus % sensitivity	Enterococcus % sensitivity
Ampicillin	-	-	40.90%
Ampicillin-Sulbactum	25.92%	18.18%	40.90%
Amikacin	81.48%	63.63%	-
Clindamycin	40.74%	9.09%	-
Cefoxitin	48.14%	9.09%	
Doxycycline	81.48%	90.90%	68.18%
Erythromycin	33.33%	18.18%	-
Gentamicin	22.22%	27.27%	59.09%
Levofloxacin	11.11%	36.36%	27.27%
Vancomycin	77.77%	100%	77.27%
Teicoplanin	77.77%	100%	77.27%
Linezolid	-	-	100%*
Minocycline	-	-	100%*