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Indian Journal of Microbiology Research

Journal homepage: <https://www.ijmronline.org/>

## Original Research Article

## Prevalence of MRSA, ESBL, and AMPC-beta-lactamase-producing bacterial profile in pus sample

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## ARTICLE INFO

## Article history:

Received 02-12-2022

Accepted 21-12-2022

Available online 23-01-2023

## Keywords:

Antibiotic

Resistance

AMPC β- Lactamase

ESBL

Bacteria

Pus

## ABSTRACT

**Introduction:** Rapid distribution of bacteria in wound infection and their appropriate treatment with antibiotics are crucial for health care providers. Bangladesh is vulnerable to multi-drug-resistant antibiotics due to extensive antibiotic misuse and other factors. The purpose of the present study was to see the frequency and distribution of bacteria isolated from pus and sensitivity patterns among hospitalized patients.

**Materials and Methods:** A cross-sectional study was carried out from June 2020 to July 2021. The pus samples were collected from the patients who visited BIHS General Hospital, Dhaka. Isolation and Identification of bacteria were made by culture and biochemical test and antibiotic susceptibility test was done by disc diffusion method.

**Results:** The most common isolates were *S.aureus*. Gram-positive bacteria were mostly resistant to Penicillin, Cefoxitin, Ampicillin, Azithromycin, Cotrimoxazole, Cefuroxime, and Cepradine antibiotics. 57.1% of *S.aureus* and (100%) of *S.epidermidis* was Methicillin-resistant, AMPC β-Lactamase producing bacteria (52.2%), and ESBL are (13.0%).

**Conclusion:** Due to the abuse of antibiotics, Methicillin-resistant AMPC β-Lactamase, ESBL are increasing day by day. Our study found that MDR bacteria is increasing rapidly and which is a major problem. Therefore, Antibiotic susceptibility pattern testing is required before the use of antibiotics, and continuous monitoring of antibiotic sensitivity is needed to minimize resistance.

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## 1. Introduction

Wound infection is a common source of morbidity. Prolonged hospitalization is one of the side consequences of such infections therefore; infection control is a complicated and crucial element of wound care,<sup>1</sup> most importantly in light of the world's rising antimicrobial resistance problem.<sup>2</sup> During or after trauma, burn injuries, or surgical procedures, microbial pathogens cause human skin and soft tissue infections (SSTI), which culminate in the creation

of pus, a white to the yellow fluid containing dead WBC, cellular debris, and necrotic tissues.<sup>3</sup> *Staphylococcus aureus* (*S.aureus*), *Klebsiella pneumoniae*, *Pseudomonas*, *Escherichia coli*, and *Streptococci* are the most prevalent pus-producing bacteria, with *S.aureus* being the most prevalent.<sup>4</sup> These bacteria, linked to wound infections, are widespread in hospitals and cause severe morbidity, and a considerable financial burden to human life, investigating virulence and antibiotic sensitivity, a pure bacterial culture is required.<sup>5</sup> To limit the infection that occurs in wound infection, an effective antimicrobial treatment needs knowledge of the prospective microbial

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pathogen.<sup>6</sup> Antibiotic-resistance results from misuse of drugs and its fast spread among dangerous bacterial isolates, regarded as severe concerns to public health worldwide. Studies revealed that blended antibiotics could be effective.<sup>7</sup> The emergence of antibiotic-resistance is a severe threat to human life.<sup>8</sup> With a rapidly declining pipeline of new antibiotics, this emergence of multi-drug-resistant, pathogenic organisms raises serious concerns about patient and public health.<sup>9</sup> ESBL manufacturing strains are most likely additional current than is presently recognized as a result of they typically stay unseen by routine status testing methods. ESBL strains are related to resistance to different non  $\beta$ -lactam antibiotics just like the aminoglycosides and Chloramphenicol.<sup>10</sup> Knowing about the bacterial isolates on time-to-time and finding the antibiotic-resistant pattern is a burning issue for microbiologists. Consequently, a severe issue like multi-drug resistant will also be focused on by the doctors.

The focus of this research, investigate the frequency and resistance of the bacterial isolates from pus sample collected from BIHS General Hospital, to find out the types and frequency of isolates & the antibiotic-resistance of them.

## 2. Materials and Methods

For this research, a Cross-Sectional Study design, purposive sample technique was selected, conducted in the Microbiology Laboratory of BUHS, Darus Salam, Mirpur Dhaka. The study period was 12 months from 1st June 2020 to 31st July 2021.

A questionnaire used for the collection of data. Data on age, sex etc. were collected. 50 Pus samples collected from the Microbiology Department, BIHS General Hospital. After collecting pus samples from the laboratory, the samples were labeled correctly and transported maintaining 2–4° C temperature without any delay to the laboratory of the Microbiology Department, BUHS.

Collected pus was processed for Gram-staining and culturing. The samples were aseptically inoculated on Blood agar or Brain Heart Infusion agar and MacConkey's agar plates incubated aerobically at 35°C–37°C for 24–48 hrs. Identification and characterization of isolates were performed based on standard microbiological methods.

The antimicrobial-susceptibility of all selected isolates had been tested through a standardized double-disk diffusion method known as Kirby-Bauer. Antibiotic condition testing will screen for ESBL production by noting specific zone diameters that indicate a high level of suspicion for ESBL production by mistreatment Cefuroxime, Ceftazidime, Aztreonam, Cefotaxime, or Ceftriaxone. The CLSI has planned disc diffusion ways for screening for ESBL production by *Klebsiella*, *E.coli*, and *Proteus spp.* If any of the zone diameters indicate suspicion for ESBL production, makeup validator tests ought to be accustomed ascertain the diagnosis.<sup>11</sup>

Techniques to spot AmpC  $\beta$ -lactamase-producing isolates are obtainable however are still evolving and don't seem to be nonetheless optimized for the clinical laboratory, that results in the underestimate of those resistance mechanisms. Carbapenemase will typically be accustomed treat infections because of AmpC-producing bacteria, but carbapenem resistance can arise in some organisms by mutations that cut back flow (outer membrane porin loss) or enhance effluence (efflux pump activation).<sup>12</sup>

### 2.1. Statistical analysis

Data was input on Microsoft excel, and statistical analysis was done by using SPSS V16.

### 2.2. Quality control

Quality control was done by using control organism *Escherichia coli* ATCC 25922 and *Staphylococcus* ATCC 29213.

### 2.3. Ethical consideration

This study reviewed by the ethical review committee of BUHS (Memo No: BUHS/ERC/EA/21/277).

## 3. Result

Pus was collected from 50 patients of which, were indoor patients 18(36%), and outdoor patients 32(64%). Among 50 patients majority were in the age group of 40 to 50 years, 16(32%). Males, 28(56%), were more predominant than females, 22(44%). The culture was positive in the majority, 38(76%), of the samples. The predominant bacteria were *S. aureus*, followed by *Klebsiella pneumoniae* and *E.coli*. *S.aureus* was found to be highly resistant to Penicillin, Cefoxitin, Ampicillin, Azithromycin, Cotrimoxazole, Cefuroxime, Cepradine and less resistant to Amikacin, Gentamicin. Among 14 *S.aureus* were MRSA while a single *S.epidermidis* 8(100%) found MRSS, and 3(21.1%) were VRSA. *Klebsiella pneumoniae* was 100% resistant to Cepradine and Cefuroxime, followed by Cotrimoxazole (85.71%), and Cefixime (71.42%). In the case of gram-negative bacilli, 12(52.2%) were AMPC  $\beta$ -Lactamase positive, 3(13.0%) ESBL positive and 2(8.7%) were both AMPC  $\beta$ -Lactamase & Carbapenemase positive. Table 1, showed males were predominant in study subjects 28(56%) followed by female 22(44%). Table 2, showed that among 50 patients, majority were in the age group of 40 to 50 years(32%) followed by <40 years and > 60 years both of which were(24%) and age group 50-60 was 10(20%). Among the male 40 to 50 Year was the most common age group 11(22%). On the other hand, among female, <40 year was highest 8(16%) age group. Table 3, showed, Out of 50 samples, 38(76%) showed growth and 12(24%) no growth. Table 4, showed out of 50

samples, predominant samples were from OPD, 32(64%) followed by IPD, 18(36%). Table also shows that growth of bacteria was predominant in samples from OPD, 24(63.2%) followed by IPD, 14(36.8%). Table 5, showed that both gram-positive bacteria (73.3%) and gram-negative bacteria (56.5%) were predominant in OPD. Table 6, showed distribution of bacteria in study population. Total 8 types of bacteria were isolates from pus sample. Among gram-positive isolates most prevalent bacteria is *S.aureus* (n=14). whereas, among gram negative isolates most prevalent isolates is *Klebsiella pneumoniae* (n=7). Table 7, showed distribution of gram positive bacteria based on OPD & IPD. Out of 15, predominant bacteria was *S.aureus* 14(93.4%) of which 10(66.7%) was from outdoor patients and 4(26.7%) from indoor patient. On the other hand, Only 1(6.7%) was *S.epidermidis* which was isolated sample from samples of outdoor patients. Table 8, showed distribution of gram negative bacteria in indoor and outdoor patients. *Klebsiella pneumoniae* was the predominant bacteria 7(30.4%) of which 6(26.1%) was from outdoor patients and 4.3% from indoor patients. The next highest organism found was *E. coli* 4(17.3%) of which 3(13%) was from outdoor and rest 1(4.3%) from indoor patients. However, each of *Proteus*, *Enterobacter*, *Acinetobacter*, *Pseudomonas* was equally (13%) isolated from the samples. Proportion of *Proteus* was predominantly from outdoor (8.7%). But *Enterobacter* 8.7%, *Acinetobacter* 13%, and *Pseudomonas* 8.7% were predominant in indoor samples.

**Table 1:** Distribution of gender among study population (n=50)

Gender	Frequency	Percentage
Male	28	56%
Female	22	44%
Total	50	100%

**Table 2:** Distribution of age and sex among study population (n=50)

Gender	<40 yrs	40-50 yrs	50-60 yrs	>60 yrs	Mean & SD
Male	4(8%)	11(22%)	6(12%)	7(14%)	Mean= 50.4
Female	8(16%)	5(10%)	4(8%)	5(10%)	SD= 13.7
Total	12(24%)	16(32%)	10(20%)	12(24%)	50(100%)

**Table 3:** Frequency of isolates in study population (n=50)

Culture	Frequency	Percentage
Growth	38	(76%)
No-growth	12	(24%)
Total	50	(100%)

Table 9, showed distribution of antibiotic-resistance pattern of gram positive isolates. *S. aureus* showed highest resistance to penicillin(100%) followed by (92.85%) to Ampicillin, Azithromycin, Cefuroxime, Cepradine,

**Table 4:** Distribution of isolates based on IPD (Indoor) and OPD (outdoor) department

Department	Frequency	
	Pus Sample	Bacteria
IPD	18(36%)	14(36.8%)
OPD	32(64%)	24(63.2%)
Total	50(100%)	38(100%)

**Table 5:** Distribution of bacteria according to Gram stain and IPD & OPD

Gram stain	IPD	OPD	Total
Gram Positive	4(26.7)	11(73.3%)	15(100)
Gram Negative	10(43.5%)	13(56.5%)	23(100)
Total	14(36.8%)	24(63.2%)	38(100)

**Table 6:** Distribution of bacteria in study population

Gram stain.	Name of Bacteria	Proportion
Gram positive Bacteria (n=15)	<i>S.aureus</i> (n=14)	93.4%
	<i>S.epidermidis</i> (n=1)	6.6%
	<i>Proteus spp.</i> (n=3)	13%
	<i>Klebsiella pneumoniae</i> (n=7)	30.4%
Gram Negative Bacteria (n=23)	<i>Enterobacter spp.</i> (n=3)	13%
	<i>E.coli</i> (n=4)	17.3%
	<i>Acinetobacter spp.</i> (n=3)	13%
	<i>Pseudomonas spp.</i> (n=3)	13%

Cotrimoxazole. It showed lowest resistance to Gentamicin, Amikacin, Cefixime and Cefotaxime. However, *S.aureus* showed variable degree of resistance to other antibiotics, Ciprofloxacin 12(85.71%), Clindamycin 11(78.57%), 10(71.43%) Amoxiclav, Doxycyclin, Cefoxitin 8(57.14%), and Ceftazidime 7(50%). Single *S.epidermidis* was found 100% sensitive to Amikacin and Gentamicin and 100% resistant to all other antibiotics.

Table 10, showed antibiotic resistance pattern of gram negative isolates. *Klebsiella pneumoniae* was 100% resistant to Cepradine and Cefuroxime, followed by 6(85.71%) Ampicillin, Tetracycline, Cotrimoxazole, (71.42%) to Amoxiclav, Cefixime, Cefotaxime, Ceftazidime. However, *Klebsiella pneumoniae* was 100% sensitive to Amikacin and Imipenem followed by also 85.7% sensitive to Tigecycline and Colistin. This organism showed variable degree of resistant against other antibiotics. *E.Coli*, showed 100% resistance to Amoxiclav, Cefixime, Cefuroxime, Cepradine, Cefotaxime, Ceftadizime followed by Ampicillin, Aztreonam, Cotrimoxazole which showed 75% each. However, *E.coli* was 100% sensitive to Amikacin, Gentamicin, Tigecycline and 75% sensitivity to Imipenem. In *Enterobacter spp.* was highly resistant (66.7%) to each of antibiotics. It was 100% sensitive to Amikacin, Colistin, Gentamicin and Tigecycline. *Acinetobacter*, *Pseudomonas* were estimated 100% resistant to various antibiotics. These two organisms

**Table 7:** Distribution of species of gram positive bacteria based on IPD (Indoor) and OPD (outdoor) department

Gram stain	Organism	Indoor patient	Outdoor patient	Total
Positive (n=15)	<i>S.aureus</i>	4(26.7%)	10(66.7%)	14(93.4%)
	<i>Staphylococcus epidermidis</i>	0(0%)	1(6.6%)	6.6%

**Table 8:** Distribution of gram-negative bacteria on basis of IPD (Indoor) and OPD (Outdoor) department

Gram stain	Organism	Indoor patient	Outdoor patient	Total
Negative (n=23)	<i>Proteus</i>	1(4.3%)	2(8.7%)	13%
	<i>Klebsiella pneumoniae</i>	1(4.3%)	6(26.1%)	30.4%
	<i>Enterobacter</i>	2(8.7%)	1(4.3%)	13%
	<i>E.coli</i>	1(4.3%)	3(13%)	17.3%
	<i>Acinetobacter</i>	3(13.0%)	0(0%)	13%
	<i>Pseudomonas</i>	2(8.7%)	1(4.3%)	13%
	Total		10(43.5%)	13(56.5%)

**Table 9:** Antibiotic resistance pattern in gram-positive isolates

Antibiotics	<i>S. aureus</i> (n=14)	<i>S. Epidermidis</i> (n=1)
Amikacin	1(7.12%)	0(0.00%)
Ampicillin	13(92.85%)	1(100%)
Amoxiclav	10(71.43%)	1(100%)
Azithromycin	13(92.85%)	1(100%)
Cefixime	5(35.71%)	1(100%)
Cefuroxime	13(92.85%)	1(100%)
Cephradine	13(92.85%)	1(100%)
Cefotaxime	5(35.71%)	1(100%)
Ceftazidime	7(50.00%)	1(100%)
Ciprofloxacin	12(85.71%)	1(100%)
Cotrimoxazole	13(92.85%)	1(100%)
Cefoxitin	8(57.14%)	1(100%)
Clindamycin	11(78.57%)	1(100%)
Doxycyclin	10(71.43%)	1(100%)
Gentamicin	2(14.28%)	0(0.0%)
Vancomycin	3(21.4%)	0(0.00)
Linezolid	9(64.3%)	0(0.00%)
Penicillin	14 (100%)	1(100%)

showed verities of sensitivity to Colistin and Imipenem. *Proteus* showed 100% resistant to Ampicillin, Cefuroxime, Cephradine, Cotrimoxazole, Colistin, Tetracycline, in addition 100% sensitivity to other antibiotics.

Note: MRSA= Methicillin-Resistant *Staphylococcus aureus*, MRSS= Methicillin-Resistant *Staphylococcus Species.*, VRSA= Vancomycin-Resistant *Staphylococcus aureus*, VRSS= Vancomycin-Resistant *Staphylococcus Species.*

Table 11, showed out of 14 *S.aureus*, 8(57.1%) was resistant Methicillin (MRSA). However, the single *S.epidermidis* was resistant (100%) to methicillin(MRSS). On the other hand, *S.aureus* 3(21.1%) resistance to Vancomycin and *S.epidermidis* 0% to Vancomycin.

Table 12, indicated the list and percentage of enzymes produced from gram negative bacteria, responsible for drug resistance. A high amount of AMPC was produces from 12(52.2%) organisms followed by (ESBL) 3(13.0%) and

**Table 11:** Isolates resistant to methicillin

Organism	MRSA	MRSS	VRSA/VRSS
<i>S.Aureus</i> (n=14)	8(57.14%)	-	3(21.1%)
<i>S.Epidermidis</i> (n=1)	-	1(100%)	0(0%)

AMPC  $\beta$ -Lactamase & Carbapenemase together 2(8.7%).

**Table 12:** Production of Enzymes responsible for drug resistance (n=23)

Name of enzymes	Percentage
AMPC $\beta$ -Lactamase	12(52.2%)
Extended-spectrum $\beta$ -Lactamase (ESBL)	3(13.0%)
AMPC $\beta$ -Lactamase & Carbapenemase	2(8.7%)

Table 13, showed in present study among 23 the isolates, 12(52.2%) bacteria were AMPC  $\beta$ -Lactamase

**Table 10:** Antibiotic resistance pattern in gram negative bacteria

Antibiotics	<i>Proteus</i> (n=3)	<i>Klebsiella</i> <i>Pneumoniae</i> (n=7)	<i>Enterobacter</i> <i>SPP</i> (n=3)	<i>E.coli</i> (n=4)	<i>Acinetobacter</i> (n=3)	<i>Pseudomonas</i> (n=3)
Amikacin	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(33.3%)	1(33.3%)
Ampicillin	3(100%)	6(85.7%)	1(33.3%)	3(75%)	3(100%)	3(100%)
Amoxiclav	1(33.3%)	5(71.4%)	2(66.7%)	4(100%)	3(100%)	3(100%)
Aztreonam	0(0.0%)	4(57.1%)	2(66.7%)	3(75%)	3(100%)	3(100%)
Cefixime	0(0.0%)	5(71.4%)	2(66.7%)	4(100%)	3(100%)	3(100%)
Cefuroxime	3(100%)	7(100%)	2(66.7%)	4(100%)	3(100%)	3(100%)
Cephradine	3(100%)	7(100%)	2(66.7%)	4(100%)	3(100%)	3(100%)
Cefotaxime	0(0.0%)	5(71.4%)	2(66.7%)	4(100%)	3(100%)	3(100%)
Ceftazidime	0(0.0%)	5(71.4%)	2(66.7%)	4(100%)	3(100%)	3(100%)
Cotrimoxazole	3(100%)	6(85.7%)	2(66.7%)	3(75%)	3(100%)	3(100%)
Colistin	3(100%)	1(14.3%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Ciprofloxacin	1(33.3%)	4(57.1%)	1(33.3%)	3(75%)	2(66.7%)	2(66.7%)
Gentamicin	0(0.0%)	1(14.3%)	0(0.0%)	0(0.0%)	1(33.3%)	1(33.3%)
Imipenem	0(0.0%)	0(0.0%)	0(0.0%)	1(25%)	1(33.3%)	0(0.0%)
Tetracycline	3(100%)	6(85.7%)	2(66.7%)	2(50%)	2(66.7%)	3(100%)
Tigecycline	0(0.0%)	1(14.3%)	0(0.0%)	0(0.0%)	0(0.0%)	3(100%)
Cefoxitin	0(0.0%)	4(57.1%)	2(66.7%)	2(50%)	3(100%)	3(100%)

producer, 3(13.0%) ESBL producer and 2(8.7%) both of AMPC  $\beta$ -Lactamase & Carbapenemase producers. Out of 7 *Klebsiella spp.* 4 was AMPC  $\beta$ -Lactamase producer, an ESBL producer. Out of 4 *E.coli* 1(25%) AMPC  $\beta$ -Lactamase producer, 2(50%) ESBL producer, and 1(25%) both AMPC  $\beta$ -Lactamase and Carbapenemase producers. 3(100%) *Pseudomonas spp.* found AMPC  $\beta$ -Lactamase producers. Followed by *Acinetobacter spp.* and *Enterobacter spp.* 2(66.7%) each. *Proteus spp.*, *Enterobacter spp.*, *Acinetobacter spp.* (n=3), *Pseudomonas spp.* (n=3) found non ESBL producer.

#### 4. Discussion

As wound infection becomes the most common hospital-acquired illness, the hospital environment plays vital role in wound infection (Khanam et al.2018).<sup>13</sup> In present study, out of 50 participants, majority were male followed by females, similar with (Batra et al.2020)<sup>4</sup> where males were predominant 1116(58.6%) than females. In this study, the mean of age group was approximately 50 years (Karmaker et al 2016).<sup>14</sup> In our study, out of 50 pus samples (n=50), which collected predominantly from OPD (64%), followed by IPD (36%), majority (76%) were growth positive (Trojan et al 2016).<sup>3</sup> Frequency of gram-positive bacteria and gram-negative bacteria in this study was 15(39.5%) and 23(60.5%). Comparing with (Rai et al 2017)<sup>15</sup> where out of 264 growth positive samples, gram-positive was 61% followed by gram-negative bacteria 39%. In our study, out of 15 gram positive cocci, predominant bacteria was *S.aureus* 14(93.4%) of which 10(66.7%) was from outdoor patients and 4(26.7%) from indoor patient. On the other hand, only 1(6.7%) was *Staphylococcus epidermidis*,

which was isolated from samples of outdoor patients. Another study of (Wadekar et al 2020),<sup>16</sup> among gram 39 gram-positive cocci, predominant organism was *S.aureus* (69.2%) followed by *Cons* (25.6%) and *Enterococci* (5.2%). However, (Rai et al.2017),<sup>15</sup> found out of 162 gram-positive isolates, where 99% was *S.aureus* followed by *S.pyogenes* 2%. This study demonstrate the prevalence of gram-negative bacteria (n=23) where *Klebsiella pneumoniae* (30.4%) was the predominant organism followed by *E.coli* (17.3%), *Proteus spp.*(13%), *Enterobacter spp.* (13%), *Acinetobacter* (13%) and *Pseudomonas* (13%) isolated from pus. Study by (Wang et al 2018)<sup>17</sup> found *Klebsiella* (80.3%), the most commonly occurring pathogens in liver abscess infections followed by *E.coli* (7.8%), *P.aeruginosa* (1.9%), and *Acinetobacter baumannii*(1%). Thanni et al.(2003)<sup>1</sup> found that *Pseudomonas spp.* was (29.9%), *Klebsiella spp.*(18.5%), *Proteus spp.*(15.1%), and *E.coli* (7%). Serraino et al 2018)<sup>18</sup> found *E. coli* (26.5%), *Klebsiella Spp.* (5.6%), *Proteus Spp.* (1.9%) and *Citrobacter spp.* (1.9%) to be the most common pathogen with similar observations. *S.aureus* showed highest resistance to penicillin (100%), Ampicillin, Azithromycin, Cefuroxime, Cephradine, Cotrimoxazole showed (92.85%). However, *S.aureus* showed variable degree of resistance to other antibiotics, comparing with (Rai et al 2017),<sup>15</sup> out of 160 *S.aureus*, they also found resistance range from varieties antibiotics, similar with (Wadekar et al 2020).<sup>16</sup>Single *S.epidermidis*, was found (100%) sensitive to Amikacin and Gentamicin, also (100%) resistant to all other antibiotics. Out of 10 CoNS, (20%) resistant to Amikacin and (40%) to Gentamicin. They also showed variable resistance. In this study, *Klebsiella pneumoniae* showed 100% resistant to Cephradine and Cefuroxime. However, it

**Table 13:** ESBL, AMPC and Carbapenemase production pattern in gram negative isolates

Organism	AMPC $\beta$ -Lactamase	ESBL	AMPC $\beta$ -Lactamase & Carbapenemase	Total
Proteus spp.(n=3)	0(0.0%)	0(0.0%)	0(0.0%)	0.0%
Klebsiella spp.(n=7)	4(57.1%)	1(14.3%)	0(0.0%)	5(71.4%)
Enterobacter spp.(n=3)	2(66.7%)	0(0.0%)	0(0.0%)	2(66.7%)
E.coli(n=4)	1(25%)	2(50%)	1(25%)	4(100%)
Acinetobacter spp.(n=3)	2(66.7%)	0(0.00%)	1(33.3%)	3(100%)
Pseudomonas spp.(n=3)	3(100%)	0(0.0%)	0(0.0%)	3(100%)
Total(23)	12(52.2%)	3(13.0%)	2 (8.7%)	17(73.9%)

showed lowest resistance (0%) to Amikacin and Imipenem. This organism showed variable degree of resistance against other antibiotics. Another study by (Roy et al 2017)<sup>19</sup> and (Khatun et al 2020)<sup>20</sup> found similar antibiotic-resistance pattern of *Klebsiella spp. E.Coli*, which showed (100%) resistance to each of Amoxiclav, Cefixime, Cefuroxime, Cepradine, Cefotaxime, Ceftadizime. This bacteria also showed a variety of sensitivity to other antibiotics which related to (Khalid et al 2019),<sup>21</sup> where they found their antibiotics result as similar as like our result. In *Enterobacter spp.* high resistance (66.7%) was seen to each of antibiotics we use. *Pseudomonas* was 100% resistant to maximum antibiotics, it showed no resistance to Colistin and Imipenem, contrast with (Khatun et al.2020),<sup>20</sup> where (75%) resistance of Ceftadizime and Gentamicin. Another study by (Paudel et al 2021)<sup>22</sup> showed various range of antibiotic-resistance, dissimilarity with the present study. *Acinetobacter* showed variable degree of resistance against Tetracycline (66.7%), Amikacin, Gentamicin and Imipenem (33.3%) and 0% resistance to Tigecycline and Colistin, along with (100%) resistant to other antibiotics. Where (Khanom et al 2018),<sup>13</sup> showed (87.5%) resistance to Imipenem, (90%) to Gentamicin, (50%) to Cotrimoxazole. *Proteus* showed (100%) resistant to Ampicillin, Cefuroxime, Cepradine, Cotrimoxazole, Colistin, Tetracycline, also found similar with Roy et al (2017).<sup>19</sup> In our study, among gram-positive bacteria, out of 14 *S.aureus*, 8(57.1%) was Methicillin resistant (MRSA). However, the single *S.epidermidis* was (100%) Methicillin resistant (MRSS). 3(21.1%) *S.aureus* was Vancomycin resistant (VRSA) and (0%) *S.epidermidis* was Vancomycin resistant (VRSS). In present study, among 23 gram native bacteria, 12(52.2%) were AMPC  $\beta$ -Lactamase producer, 3(13.0%) ESBL producer and 2(8.7%) both of AMPC  $\beta$ -Lactamase & Carbapenemase producers. 100% *Pseudomonas spp.* found AMPC  $\beta$ -Lactamase producers followed by *Acinetobacter spp.* and *Enterobacter spp.* (66.7% each), *Klebsiella spp.* (57.1%), and *E.coli* 25%. *Proteus spp.*, *Enterobacter spp.*, *Acinetobacter spp.* (n=3), *Pseudomonas spp.* (n=3) found non ESBL producer. (Upreti N et al. 2018)<sup>2</sup> have reported 13.51% and 16.55% of *E. coli* and *Klebsiella* as ESBL producer respectively. Another study by (Wang et al. 2018)<sup>17</sup> revealed that 5

strains of *E coli* produced extended-spectrum  $\beta$ -Lactamase (ESBL) which correlates with the present study.

## 5. Conclusion

The most common isolated bacteria after aerobic culture of Pus was *Staphylococcus aureus* among gram-positive bacteria. The Gram-negative bacilli *Klebsiella pneumoniae* and *E.coli* were the most common bacteria causing wound infection. This study also outlines the antibiotic-resistance of bacterial isolates, which will help formulate the local antibiotic policy for the hospital and start the appropriate empirical antibiotic treatment before the culture reports are available.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

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**Cite this article:** Quayum A, Karmaker M, Yesmin Ananna T, Asna SMZH. Prevalence of MRSA, ESBL, and AMPC-beta-lactamase-producing bacterial profile in pus sample. *Indian J Microbiol Res* 2022;9(4):272-278.