RESEARCH PAPER

Antibiotic susceptibility trends in bacterial isolates from wound infections

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ABSTRACT

Pyogenic infections leading to pus formation are associated with various inflammatory mediators and the proliferation of microbial flora. Wound infections can be caused by both aerobic and anaerobic bacteria, viruses, fungi, or even protozoans. Timely treatment of infected patients is critical since untreated or poorly treated pyogenic infections can lead to mortality or severe complications. Successful treatment is defined by choosing the right antibiotic, which is hindered by the spreading of bacterial drug resistance. It is well documented that overuse of antimicrobials before and especially during the COVID-19 pandemic has resulted in the emergence of drug resistance among various microorganisms. Our study aimed to identify common etiological agents associated with wound infections and to evaluate their antibiotic resistance patterns.

A cross-sectional study was conducted by analyzing the antibiograms of different aerobic bacteria isolated from wound samples of patients from the Palakkad District Hospital in Kerala, India, from 2017 through 2019. All variables, including age, sex, bacterial isolate, and antibiogram, were tabulated and the chi-square test was used for analysis. Out of the 1,269 bacterial isolates analyzed, the most common gram-negative strain was *Escherichia coli* (23%) followed by *Pseudomonas aeruginosa* (20%). Among the gram-positive isolates, *Staphylococcus aureus* was the most common (19%). We found only 15% of *Staphylococcus aureus* isolates were methicillin-resistant, against the national average of 41%. The most resistant strain in our study was *Acinetobacter baumannii*. Statistically significant changes in antibiotic resistance patterns were observed over the three years of the study period.

INTRODUCTION

Timely treatment of pyogenic infections in wounds is a primary task for the medical personnel in hospitals worldwide. These infections are associated with various pathogens, such as aerobic and anaerobic bacteria, viruses, fungi, or even protozoans, and result in inflammation and pus formation. Polymicrobial pyogenic infections are also encountered. Common etiological agents associated with wound infections include *Pseudomonas* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Escherichia coli*, *Proteus* spp., *Streptococcus* spp., *Enterobacter* spp., and coagulase-negative Staphylococci. There is a significant difference in these etiological agents between countries, though they also vary from hospital to hospital [1, 2]. In addition, there are significant variations in the antibiotic sensitivity patterns among the bacterial isolates causing pyogenic infections [3, 4, 5]. Empirical treatment of wound infections regardless of the bacterial sensitivity to the administered antibiotics has led to the emergence of resistant strains both in the community and in hospitals. The horizontal transfer of resistance genes between different bacteria adds to the gravity of this situation [6]. The available treatment options are severely limited. Moreover, the prolonged hospital stay required to treat such resistant microorganisms adds to the economic burden of the country [3]. According to the World Health Organization (WHO), resistance to first-line antibiotics varies from zero to 100%. In some cases, bacterial strains are resistant to second- and third-line drugs [7]. It has now become a current opinion that coordinated action must be ensured to address antimicrobial resistance [8]. There is an urgent need to treat infections based on the antibiotic sensitivity pattern. In addition, each hospital must have its own antibiotic policy and a system for continuous monitoring of drug susceptibility of bacterial isolates.

This project aimed to identify the etiological agents associated with wound infections in Kerala state and their antibiotic resistance patterns. A literature search revealed no corresponding studies from this part of Kerala. Our results can help to develop a tailored antimicrobial policy for the Palakkad District Hospital.

MATERIALS AND METHODS

A cross-sectional study (Study ID: IEC/GMCPKD15/19) was performed by analyzing the antibiogram data of different aerobic bacteria isolated from wound samples (diabetic ulcers, post-operative wounds, burn wounds, and other wounds) obtained in the Microbiology laboratory of the Palakkad District Hospital over three years (2017-2019). All the data, including the patients' age and sex, bacterial isolates, and antibiograms, were entered into an Excel sheet. The study was approved by the Institutional Research Board and the Ethical Committee of the hospital (on July 22, 2019). Data were analyzed using SPSS version 20 software. The chi-square test was used for statistical analysis.

RESULTS

We received a total of 1,843 pus samples in the laboratory over three years (2017-2019). Among these, 571 samples showed no growth of aerobic bacteria and were excluded from the study. Another 124 samples yielded mixed bacterial growth with >3 different types of organisms and were not processed further. The remaining 1,148 samples were included in our study. Culture positivity was 69.0%. From these 1,148 samples 1,027 yielded one type of bacteria while from the rest 121 samples two different bacterial species were isolates. Therefore, we investigated totally 1,269 bacterial isolates in the course of our study. We did not isolate or analyze anaerobic bacteria, since anaerobic culture is not routinely performed in our laboratory.

The male-to-female ratio was 1.48:1. The majority of the samples came from the 51-60 age group followed by the 41-50 age group: 246 (21.4 %) and 226 (19.7%), respectively. Out of the 1,148 samples that were included in the study, 416 samples came from patients who visited the outpatient department, and 732 samples were obtained from patients admitted to the wards.

The most common gram-negative bacterial isolate was *Escherichia coli* (289 isolates, 23%) followed by *Pseudomonas aeruginosa* (249, 20%). Among the gram-positive isolates, methicillin-sensitive *Staphylococcus aureus* (MSSA) was the most common (199, 16% of all isolates), followed by methicillin-resistant *Staphylococcus aureus* (MRSA) (36, 3% of all isolates). The distribution of gram-positive and gram-negative bacteria is shown in Fig. 1, while the frequency of microorganism occurrences is shown in Fig. 2.

The predominant microorganisms associated with polymicrobial infection were *Klebsiella pneumoniae/ Pseudomonas aeruginosa* (14.9% of polymicrobial infections), followed by *E. coli/Pseudomonas aeruginosa* (12.4%), and *Klebsiella pneumoniae/E. coli* (10.7%).
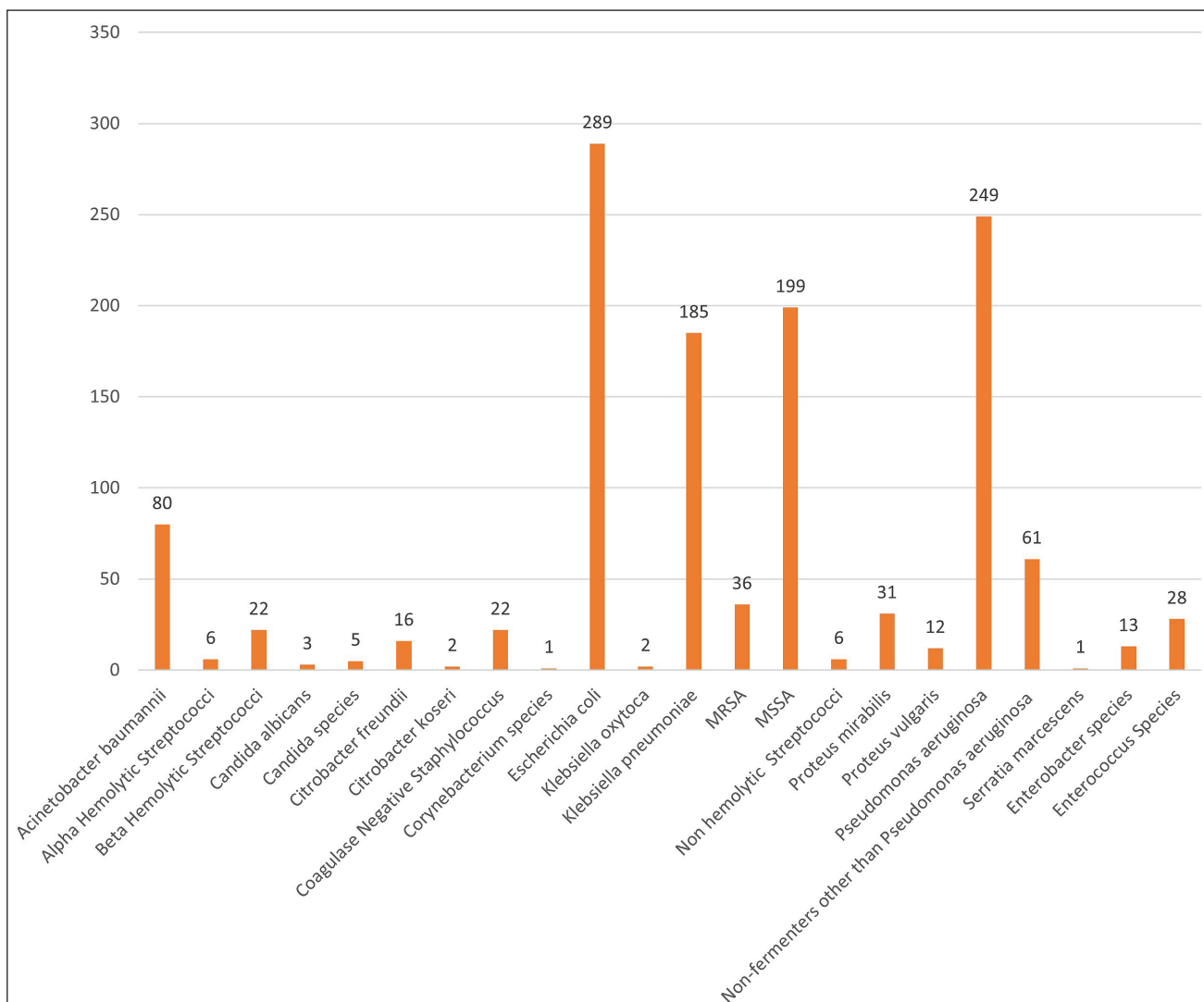
The resistance pattern of common isolates is presented in Fig. 3, Table 1, and Table 2. *E. coli* strains were most resistant to ampicillin (97.2%), as well as first- and third-generation cephalosporins (84.8% and 74.7%, respectively), and least resistant to cefoperazone-sulbactam (9.3%), imipenem, and meropenem (3.5%). All
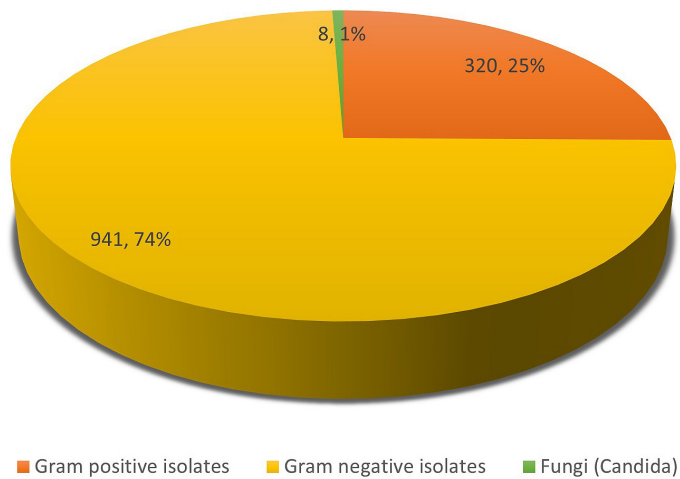
Fig. 1. Distribution of gram-negative and gram-positive bacterial isolates.

of the *Klebsiella pneumoniae* strains showed resistance to ampicillin while the most strains of this bacteria were resistant to cephalexin (81.6%) and cotrimoxazole (51.9%), and the least resistant to imipenem and meropenem (6.5%). The majority of *Proteus mirabilis* isolates were resistant to ampicillin (90.3%) followed by cephalexin (64.5%) and ciprofloxacin (41.9%). No resistance to cefoperazone-sulbactam, piperacillin-tazobactam, imipenem, or meropenem was observed in *Proteus mirabilis* isolates.

In the present study, resistance to ciprofloxacin was observed in 34.5% of *P. aeruginosa* isolates, 67.1% of *E. coli* isolates, and 49.7% of *Klebsiella pneumoniae* isolates. Resistance to gentamicin was also lower among *Pseudomonas aeruginosa* isolates (29.3% resistant strains) compared to more than 40% resistance in *E. coli* and *Klebsiella pneumoniae* isolates. Only around 7% of *Pseudomonas aeruginosa* strains demonstrated resistance to piperacillin-tazobactam, cefoperazone-sulbactam, imipenem, and meropenem. On the other hand,

Fig. 2. Distribution of bacterial isolates. Y axis: number of isolates.
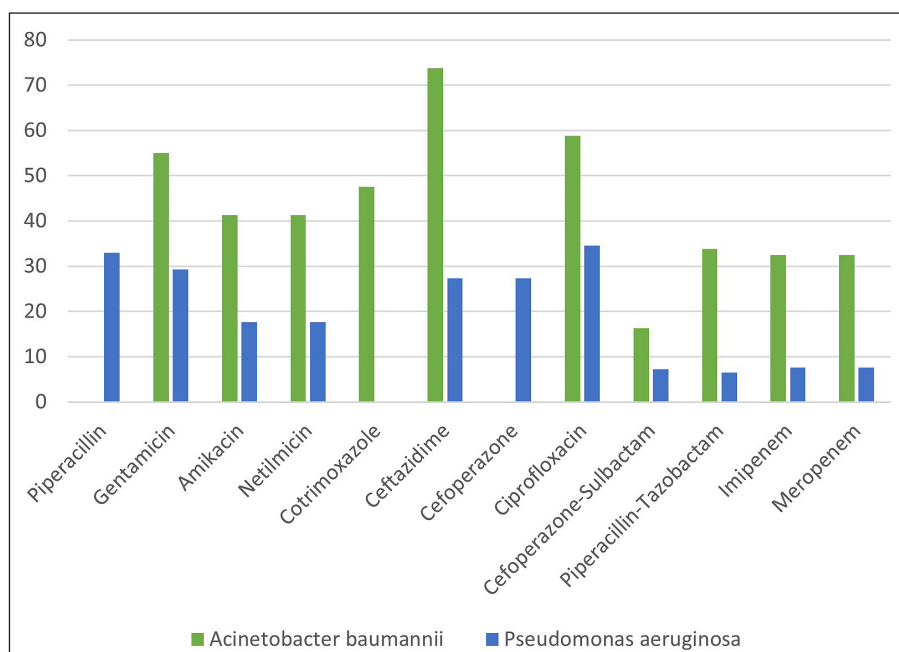
Fig. 3. Antimicrobial resistance patterns of the isolated common non-fermenters. Y axis: % of resistant strains.

Table 1. Antimicrobial resistance pattern of Enterobacteriaceae isolates

Antibiotic	Number of resistant strains (%)		
	E. coli	*Klebsiella pneumoniae*	*Proteus mirabilis*
Ampicillin	280 (97.2)	185 (100.0)	28 (90.3)
Gentamicin	117 (40.5)	81 (43.8)	10 (32.3)
Amikacin	39 (13.6)	42 (22.7)	4 (12.9)
Cotrimoxazole	163 (56.4)	96 (51.9)	4 (12.9)
Cephalexin	245 (84.8)	151 (81.6)	20 (64.5)
Cefotaxime	216 (74.7)	60 (32.4)	11 (35.5)
Ciprofloxacin	194 (67.1)	92 (49.7)	13 (41.9)
Cefoperazone-sulbactam	27 (9.3)	34 (18.4)	0
Piperacillin-tazobactam	29 (10.0)	37 (20.0)	0
Imipenem	10 (3.5)	12 (6.5)	0
Meropenem	10 (3.5)	12 (6.5)	0

Table 2. Antimicrobial resistance pattern of gram-positive isolates

Antibiotic	Number of resistant strains (%)		
	MSSA	MRSA	Enterococcus species
Ampicillin	181 (91.0)	36 (100.0)	11 (39.3)
Penicillin	181 (91.0)	36 (100.0)	28 (100)
Gentamicin	16 (8.0)	9 (25.0)	16 (57.1)
Amikacin	2 (1.0)	2 (5.6)	12 (42.9)
Cotrimoxazole	35 (17.6)	17 (47.2)	28 (100.0)
Cephalexin	0	36 (100.0)	28 (100.0)
Cloxacillin	0	36 (100.0)	Not tested
Ciprofloxacin	119 (59.8)	32 (88.9)	17 (60.7)
Erythromycin	51 (25.6)	21 (58.3)	22 (78.6)
Clindamycin	29 (14.6)	21 (58.3)	28 (100.0)
Tetracycline	27 (13.6)	11 (30.6)	18 (64.3)
Vancomycin	0	0	0

73.8% resistance to third-generation cephalosporins (ceftazidime) was observed in *Acinetobacter baumannii* strains, which also showed maximum resistance to imipenem and meropenem (32.5%).

A total of 235 *Staphylococcus* strains were isolated, of which 36 (15.3% of *Staphylococcus aureus* strains) were MRSA. Among the MSSA strains, 91% exhibited resistance to penicillin and ampicillin, while ciprofloxacin resistance was observed in 59.8% of strains (Table 2). MRSA strains (resistant to cephalexin, penicillin, and cloxacillin) showed 88.9% resistance to ciprofloxacin and 58.3% resistance to erythromycin and clindamycin. Erythromycin resistance was observed in 78.6% of the isolated *Enterococcus* species, and 64.3% of these strains were resistant to tetracycline. At the same time all the *Enterococcus* species were resistant to cotrimoxazole, cephalexin, and clindamycin.

We analyzed the changes in antibiotic resistance patterns in outpatient and inpatient bacterial isolates over the three-year period (Table 3). Most isolates showed a stable resistance pattern, although we observed significant deviations from this trend in some bacterial strains.

Bacterial isolates from the inpatient samples demonstrated some changes in antimicrobial resistance patterns. Thus, gentamicin resistance in coagulase-negative Staphylococci declined considerably from 2018 to 2019 (p=0.021). In 2018, there was a significant decrease in the resistance to ciprofloxacin in coagulase-negative Staphylococci (p=0.021) that remained constant in 2019. A significant reduction in piperacillin-tazobactam resistance was noted among the *Pseudomonas aeruginosa* isolates over the three-year period (p=0.022), as well as a decline in resistance to imipenem and meropenem (p=0.003). Among non-fermenters other than *Pseudomonas aeruginosa,* a reduction in amikacin and netilmicin resistance was documented in 2018 (p=0.039).

Additionally, some significant changes in antimicrobial resistance patterns were observed in bacterial isolate samples received from the outpatient department. *Klebsiella pneumoniae* isolates were susceptible to cefoperazone-sulbactam and piperacillin-tazobactam in 2017 and 2018. However, in 2019, resistance to the above-mentioned antibiotics was detected in three samples (17%) from which these bacteria were isolated (p<0.05). In 2018, there was a significant decrease in ciprofloxacin resistance in *Staphylococcus aureus* (p=0.03) though in 2019 it surged more than twice. Ampicillin and cotrimoxazole resistance in *Proteus mirabilis* was the highest in 2019 (p=0.025).

DISCUSSION

The culture-positivity rate observed in our study was 69%. We did not perform the anaerobic culture analysis for the samples. In our study, the most common isolates were *E. coli* (23%), followed by *Pseudomonas aeruginosa* (20%), *Staphylococcus aureus* (19%), and *Klebsiella pneumoniae* (15%). Table 4 presents common bacterial isolates from various studies conducted in India. Thus, Patel et al. [9] and Jain et al. [10] reported that *E. coli* was the most common isolate observed in their studies. A study from Pattukkottai (Tamil Nadu state, India) showed that *Pseudomonas aeruginosa* was the most common isolate [11]. In contrast, *Staphylococcus aureus* was the most common etiological agent of wound infections in a study from Ahmedabad (Gujarat state, India) [12].

The most common bacterial combination found in polymicrobial infections in the present study was

Table 3. Significant changes in antibiotic resistance patterns observed over the years in isolates from inpatient and outpatient samples

Isolate	Antibiotic	2017	2018	2019
		Inpatient samples		
Coagulase-negative Staphylococci	Gentamicin	0	20%	0
	Ciprofloxacin	100%	40%	40%
Pseudomonas aeruginosa	Piperacillin-tazobactam	16%	4%	4%
	Imipenem / meropenem	19%	9%	0
Non-fermenters other than *Pseudomonas aeruginosa*	Amikacin / netilmicin	50%	6%	21%
		Outpatient samples		
Klebsiella pneumoniae	Cefoperazone-sulbactam	0	0	17%
	Piperacillin-tazobactam	0	0	17%
Staphylococcus aureus	Ciprofloxacin	46%	28%	62%
Proteus mirabilis	Ampicillin	0	50%	100%
	Cotrimoxazole	20%	0	75%

Klebsiella pneumoniae/Pseudomonas aeruginosa. In a study conducted in Italy, the most common combination was *Staphylococcus aureus/Pseudomonas aeruginosa* [13], while a study from Kerala state (India) published in 2015 reported the MRSA/*Pseudomonas aeruginosa* combination as the most common in polymicrobial wound infections [14].

Table 5 shows the sensitivity patterns of different isolates to common antibiotics. The percentage of sensitive strains isolated in the present study was mostly higher than that observed in other Indian medical centers [9, 10, 12, 15, 16]. According to our study, gram-negative bacterial isolates were susceptible to cefoperazone-sulbactam, piperacillin-tazobactam, imipenem, and

Table 4. Comparison of common bacterial isolates reported in studies performed in different states of India

State in India, reference	Common bacterial isolates (%)			
Kerala, present study	*Escherichia coli* (23%)	*Pseudomonas aeruginosa* (20%)	*Staphylococcus aureus* (19%)	*Klebsiella pneumoniae* (15%)
Chhattisgarh, Patel et al [9]	*Escherichia coli* (51%)	*Staphylococcus aureus* (21%)	*Klebsiella pneumoniae* (12%)	*Pseudomonas aeruginosa* (6%)
Tamil Nadu, Manikandan et al [11]	*Pseudomonas aeruginosa* (43%)	*Staphylococcus aureus* (24%)	*Staphylococcus epidermidis* (16%)	*Proteus spp.* (9%)
Tamil Nadu, Jain et al [10]	*Escherichia coli* (33%)	*Staphylococcus aureus* (19%)	*Klebsiella pneumoniae* (12%)	*Pseudomonas aeruginosa* (12%)
Gujarat, Sida et al [12]	*Staphylococcus aureus* (26%)	*Klebsiella* (23%)	*Pseudomonas aeruginosa* (18%)	*Escherichia coli* (18%)

Table 5. Comparison of antibiotic sensitivity patterns of bacterial isolates from various studies done in India

Reference	Bacterial isolate	Antibiotic sensitivity pattern (%)								
		Cot	Ak	G	Cipro	Ceft	Cefo	CPS	PT	Imi
Present study	*E. coli*	44	87	59	33	-	26	91	97	97
Sida et al [12]		17	72	-	8	-	11	61	-	72
Swain et al [16]		0	-	50	100	-	50	100	-	100
Jain et al[10]		32	76	46	21	-	21	34	36	76
Patel et al [9]		32	75	46	21	-	21	34	36	75
Saha et al [15]		53	84		79	58	11	-	5	84
Present study	*Klebsiella pneumoniae*	48	77	56	50	-	68	82	80	94
Sida et al [12]		14	25		13	-	7	31	-	43
Swain et al [16]		25	-	75	50	-	40	50	-	100
Jain et al [10]		21	50	42	42	-	0	11	42	50
Patel et al [9]		20	50	40	40	-	20	10	40	50
Saha et al [15]		30	67	-	37	26	19	-	26	67
Present study	*Acinetobacter baumannii*	53	59	45	41	27	-	84	66	68
Sida et al [12]		10	10	-	19	-	4	56	-	11
Jain et al [10]		0	0	0	0	-	0	0	0	0
Patel et al [9]		0	0	0	0	0	0	0	0	0
Saha et al [15]		0	0	-	0	0	0	-	100	0
Present study	*Pseudomonas aeruginosa*	-	82	71	66	73	-	93	94	92
Sida et al [12]		-	31	-	24	31	-	-	50	63
Swain et al [16]		-	-	67	50	33	-	50	-	83
Jain et al [10]		-	79	58	62	-	0	21	79	79
Patel et al [9]		-	80	60	60	-	0	20	80	80
Saha et al [15]		-	75	-	50	75	-	-	75	75

Abbreviations: Cot – cotrimoxazole; Ak – amikacin; G – gentamicin; Cipro – ciprofloxacin; Ceft – ceftazidime; Cefo – cefotaxime; CPS – cefoperazone-sulbactam; PT – piperacillin-tazobactam; Imi – imipenem.

aminoglycoside antibiotics. This was also noted in other studies [15, 16]. In a study performed in Puducherry by Rameshkannan et al. [17], the most common bacterial isolate was *E. coli*, and its antibiotic sensitivity pattern was similar to that observed in our study.

The highest percentage of resistant strains in our study was observed among *Acinetobacter baumannii* isolates. Similar data were reported by other authors from India and other countries. For example, extensive multidrug resistance in *Acinetobacter* strains was reported by Rugira et al. from Punjab [4]. Pandrug-resistant strains of *Acinetobacter baumannii* were also described in other Indian studies [9,10]. Furthermore, according to a Latin American study [13], carbapenem resistance can reach 90% in *Acinetobacter baumannii* isolates.

We found that *Pseudomonas aeruginosa* isolates were more sensitive to ciprofloxacin and gentamicin than *E. coli* and *Klebsiella pneumoniae*. Similar data were published by Rugira et al. [4] who found that *Pseudomonas aeruginosa* strains were more susceptible to the tested antibiotics than *Klebsiella pneumoniae*. Studies from Croatia, Canada, and Latin American countries reported a different pattern: according to their data, *Pseudomonas aeruginosa* strains were resistant to carbapenems, aminoglycosides, and ciprofloxacin [18].

Of the 235 *Staphylococcus* strains isolated in our study, 36 (15%) were MRSA. According to a multicentric study conducted in India, the prevalence of MRSA was 41% in 2013 [19]. In another study from North India, this figure reached 33.7% between 2017 and 2019 [20]. According to the Indian Council of Medical Research (ICMR) report on antimicrobial resistance, the prevalence of MRSA in India was 38.6% in 2018 and 42.1% in 2019 [21]. The frequency rate of MRSA determined in our study was lower when compared to data from other studies. Our gram-positive isolates were sensitive to vancomycin, which is similar to results published by Trojan et al. and Verma [4, 22]. We noticed a higher percentage of strains sensitive to cotrimoxazole (78%) and erythromycin (69%) among our *Staphylococcus aureus* isolates compared to other studies, where the observed sensitivity to these antibiotics was below 50% [9,10,12] (Table 6).

The overuse and misuse of antibiotics have led to the global spread of bacterial antimicrobial resistance. The prevalence of mobile genetic elements containing antimicrobial genes and horizontal gene transfer between bacteria contributed to this process significantly. It has been acknowledged in recent years that there are considerable global differences in the prevalence of antimicrobial resistance - it correlates with different

Table 6. Comparison of sensitivity patterns among *Staphylococcus aureus* isolates from various studies from India

Reference	Sensitivity pattern of *Staphylococcus aureus* (%)						
	P	**G**	**Ak**	**Cipro**	**Cot**	**Ery**	**Clin**
Present study	8	89	98	36	78	69	79
Sida et al [12]	6	65	-	66	35	43	83
Swain et al [16]	32	-	85	50	-	47	-
Jain et al [10]	11	-	-	11	34	28	73
Patel et al [9]	11	-	-	11	34	28	73
Saha et al [15]	-	-	79	74	-	47	-

Abbreviations: P – penicillin; G – gentamicin; Ak – amikacin; Cipro – ciprofloxacin; Cot – cotrimoxazole; Ery – erythromycin; Clin – clindamycin.

Table 7. Comparison of bacterial antibiotic resistance trends observed in our study with the corresponding national trends over the same period

Bacterial isolate	Antibiotic	Trend observed in the present study	National trend
Pseudomonas aeruginosa (IP samples)	Piperacillin-tazobactam	Decrease in resistance from 16% to 4% (from 2017-2019)	Overall decrease in resistance for all antipseudomonal drugs from 2016-2021
	Imipenem	Decrease in resistance from 19% to 0	
Coagulase-negative Staphylococci (IP samples)	Ciprofloxacin	Decrease in resistance from 100% to 40%	Increase in resistance by 5% during 2016-2019 (resistance in 2019 – 58%)
Klebsiella pneumoniae (OP samples)	Piperacillin-tazobactam	Increase in resistance rate from 0% to 17%	Increase in resistance rate by 4% during 2016-2019 (resistance in 2019 – 61%)
Staphylococcus aureus (OP samples)	Ciprofloxacin	Increase in resistance from 46%- 62%	Increase in resistance by 3% (resistance in 2019 – 75%)

socioeconomic factors, such as population density, sanitation practices, and others [23].

In our study, we observed very little change in resistance to antimicrobials over three years. The overall resistance rate was lower than the national average (Table 7) [21, 24]. While we observed the greater rise in resistance rates for some bacterial isolates than that reported in national data over the period of 3 years, the total percentage of resistance strains determined in our study remains lower than the national average.

Many studies have reported an increase in antimicrobial resistance over recent years [25, 26], which can be attributed to misuse and overuse of antimicrobials. Our results confirm this tendency, although a decrease in the prevalence of some resistant strains has also been observed in our study.

Continuous monitoring of antibiotic resistance over a period of 5-10 years is needed to study the changing trends in the susceptibility patterns of different microorganisms. As resistant strains continue to emerge, every hospital should formulate an antibiotic administration policy. If strictly adhered to, it can help decelerate the rate of emerging resistant strains and mitigate bacterial resistance.

LIMITATIONS OF THE STUDY

We did not isolate or analyze anaerobic bacteria, since anaerobic culture is not routinely performed in our laboratory. The ward-wise distribution of the samples could not be analyzed because the data was incomplete.

CONCLUSION

Our study identified *E. coli* as the most common bacterial isolate from wound infections. *Staphylococcus aureus* was the most commonly isolated microorganism among gram-positive bacteria. *Pseudomonas aeruginosa* isolates were more susceptible to ciprofloxacin and gentamicin than *E. coli* and *Klebsiella pneumoniae*. Gram-negative isolates were found to be mostly susceptible to cefoperazone-sulbactam, piperacillin-tazobactam, imipenem, and meropenem. The majority of *Acinetobacter baumannii* strains were resistant to most antibiotics. The prevalence of MRSA in our study was lower than the national and international average; all our gram-positive isolates were sensitive to vancomycin. We observed a few significant changes in antibiotic resistance pattern over the three years study period (2017-2019). Surveillance for a longer period might shed light on the important changes in antibiotic resistance trends.

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