

Original Research Article

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Aerobic Bacterial Isolates and their Antibiotic Susceptibility Pattern from Pus Samples in a Tertiary Care Government Hospital in Tamilnadu, India

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ABSTRACT

This prospective study was conducted in our tertiary care set up to detect the bacterial isolates with antibiotic susceptibility pattern from pus samples, along with sociodemographic factors leading to wound infections and mainly to provide relevant data of antibiotics preferred for each bacterial isolate. This would be a baseline for formulation of necessary policies for antibiotic use in our set up. In our study almost 1836 (75%) of pus samples showed bacterial growth. 1303 (71%) male patients were infected whereas only 533(29%) female patients were infected. Common age group involved is 21–40 years. Gram negative bacteria were isolated more than gram positive bacteria. Out of which *Klebsiella* species was the most predominant bacterial pathogen 635(35%) followed by *Staphylococcus aureus* 334 (18%), *Escherichia coli* 253 (14%), Coagulase negative *Staphylococcus* species 204 (11%), *Pseudomonas aeruginosa* 153 (8%), *Proteus* species 126 (7%), *Citrobacter* species 51 (3%), *Enterococcus* species 43(2%) and *Acinetobacter* species 37 (2%). Highest incidence was due to ESBL isolates of *Pseudomonas aeruginosa* 90 (59%) followed by *Klebsiella* sp 312(49%), *E. coli* 124 (49%), *Proteus* sp. 62(49%), *Acinetobacter* sp 18 (48%) and *Citrobacter* sp 18 (35%).MRSA isolates were 96(43%) in *Staphylococcus aureus* isolates followed by 84(41%) in Coagulase negative Staphylococcal species.

Keywords

ESBL, MRSA,
Bacterial pathogens
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Introduction

Skin acts as a barrier and prevents the entry of microbes into our body unless the mechanism is breached due to any injury, trauma or surgical intervention. Thus wounds formed get infected by bacterial pathogens (Rugira Trojan, 2016 and Aniruddha, *et al.*, 2015). Despite their starting point, all injuries are due to the microorganisms present inside or outside the human body or both (Maria Ayub *et al.*, 2015).

Wound infections have become a global problem in surgery due to long hospital stay,

high treatment expenditure and emergence of multidrug resistant bacteria leading to gross mutilation of patients affected (Rugira Trojan, 2016). Wound infections have become the most important cause for morbidity and mortality (Ezekiel Olugbenga Akinkunmi *et al.*, 2014) the overall incidence of wound sepsis in India is 10 – 33% (Akimoto *et al.*, 1994).

Due to the multi diversity of infection causing bacteria this study was conducted to provide relevant data which would be of immense

help to serve the patients attending our tertiary care government hospital. In spite of the progress in surgical techniques and antibiotic prophylaxis, wound infections remain the commonest postoperative complications and one of the most frequently encountered nosocomial infections worldwide (Nichols *et al.*, 2004). These lead to increasing demand for advanced surgical techniques like laparoscopy to decrease significant morbidity.

As a result of these problems, routine surveillance for hospital acquired wound infections, including wound infections, operation theatres, and critical care units is recommended by the CDC (Oluwatosin, 2005). This has become most important part of infection control policy.

Wound infections that occur postoperatively also depend on the type of surgery which is classified as clean, clean contaminated, contaminated and dirty (Lilani *et al.*, 2005). Wound infections are severe in case of contaminated and dirty surgical procedures where there is a severe breach in aseptic surgical techniques adopted due to surgery (Ezekiel Olugbenga Akinkunmi *et al.*, 2014).

The investigation of the microbiologic spectrum and antibiotic susceptibility of isolates from pus samples is therefore of increasing importance bearing in mind the increasing antibiotic resistance by microorganisms and the high incidence of surgical infections caused by these resistant organisms (Dryden *et al.*, 2005). It has been documented that the type of organisms infecting surgical wound is a reflection of the body system involved in the surgical operation. According to the reports, these organisms which are normal inhabitants of the body system usually become opportunistic pathogens when their niche is violated. Selection of an effective antimicrobial agent

for a microbial infection requires knowledge of the potential microbial pathogen, an understanding of the pathophysiology of the infectious process and an understanding of the pharmacology and pharmacokinetics of the intended therapeutic agents (Kelwin *et al.*, 1999). Both aerobic and anaerobic bacteria have been implicated in wound infections which commonly occur under hospital environment (Cerceo *et al.*, 2016).

Rapid emergence of multidrug-resistant bacteria poses a serious threat to public health globally due to the limited treatment options and late discovery of new classes of antibiotics. The incidence of MRSA (Methicillin Resistant *Staphylococcus aureus*) in India ranges from 30 to 70% (Subrata Roy, 2016).

Although *Staphylococcus aureus* is a commensal of humans, it is also a frequent cause of human infections which may become serious if caused by antimicrobial resistant strains. Antibiotic resistant *S. aureus*, especially MRSA, are equally adapted to hospitals and outer environments evolving as major pathogens of public health concern. ESBLs were found in 63.6 per cent, 66.7 per cent of *E. coli* and *K. pneumoniae* isolates (Ankur Goyal *et al.*, 2009).

Incidence of extended spectrum beta lactamase producing *Escherichia coli* among patients, healthy individuals and in the environment is 60%, 38% and 10% respectively (George *et al.*, 2014). ESBL producing strains are *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Escherichia coli* (Sanders *et al.*, 1996). The situation is serious in developing countries due to irrational prescriptions of antimicrobial agents.

The inoculum size, virulence and invasive capability of the organisms have been reported to influence the risk of infection.

Moreover, the physiological state of the tissue in the wound and immunological integrity of the host also has equal importance in determining occurrence of infection (Mengesha *et al.*, 2014).

The emergence of drug-resistant organisms in both hospitals and the community is a major concern. Surveillance studies have provided important information about changes in the spectrum of microbial pathogens and trends in the antimicrobial resistance patterns in nosocomial and community-acquired infections and continued monitoring of antimicrobial resistance patterns in hospitals is essential to guide effective empirical therapy (BN Chaudhuri *et al.*, 2007).

Focusing on this ever increasing issue of multidrug resistance due to inappropriate use of antibiotics and considering the fact that no data was available on ESBL producing GNB's and methicillin resistant *Staphylococcus aureus* in our region, the current study was undertaken in our department to provide adequate and relevant data for proper selection and use of antibiotics.

Anaerobic bacteria also form an equal part in causing wound infections. As there were no proper resources for culture of anaerobic bacteria in our facility it was not done.

The main objective of this study includes, to isolate the aerobic pyogenic bacteria from pus samples; to determine the antibiotic susceptibility pattern of gram negative and gram positive bacteria isolated from pus samples; to determine the socio demographic factors involved in this study; to provide relevant information to the clinicians of our hospital regarding the antibiotic to be prescribed to decrease the emergence and spread of multidrug resistant bacteria.

Materials and Methods

Study area

This prospective study was conducted in Diagnostic Microbiology Laboratory, Government Mohan Kumaramanalam Medical College Hospital, Salem, a tertiary care hospital situated in the centre of the city with 1971 beds and catering a minimum of 4500 patients per day from Dharmapuri, Namakkal, Krishnagiri, Hosur and districts around Salem.

Study design

This study included all the pus samples caused by wound infections received in Microbiology laboratory for bacterial culture and antibiotic susceptibility testing from both in patients and outpatients irrespective of age and sex from all the departments of this hospital.

Acceptance criteria

All the pus samples collected aseptically by sterile aspiration in sterile containers and in sterile swabs were accepted for this study

Rejection criteria

Pus samples received in unsterile containers and with containers soaked with pus due to improper closure of screw caps were rejected.

Study period

This prospective study was conducted for a period of six months from July 2016 till December 2016 in the Department of Diagnostic Microbiology in accordance with standard protocols and ethical guidelines. Direct Microscopic examination of gram stained pus smears was done. Pus cells and bacteria present were noted as per protocol.

Bacterial culture was performed by inoculation of pus aseptically onto nutrient agar, 5% sheep blood agar and MacConkey agar plates and incubated aerobically at 35–37°C for 24–48 hours. Bacterial colonies were identified and characterised based on gram staining, morphology of colonies, and biochemical reactions using standard microbiological methods (Koneman, 2006).

Bacterial suspensions were prepared for each bacterial isolate separately and turbidity matched with 0.5 McFarland Standard and lawn culture of the bacteria was performed on surface of Mueller Hinton agar plates.

Antibiotics discs containing Ampicillin (Amp) – 10 µg, Piperacillin (Pi) - 100 µg, Cefazolin (Cz) - 30 µg, Cephalexin (Cn) - 30 µg, Cefuroxime (Cxm) -30 µg, Cefixime (Cfm) -5 µg, Ceftriaxone (Ctr) -30 µg, Ceftazidime (Caz) -30 µg, Cefotaxime (Ctx) - 30 µg, Azithromycin (Azm) - 15 µg, Clarithromycin (Clr) -15 µg, Doxycycline (Do) -30 µg, Penicillin G- 100 units, Amikacin (AK) - 30 µg, Gentamicin (Gen) -10 µg, Tobramycin (Tob) -10 µg, Netilmicin (Net) -10 µg, Cotrimoxazole (Cot) -1.25/23.75 µg, Ciprofloxacin (Cip) -5 µg, Levofloxacin (Le) -5 µg, Gemifloxacin (Gem) -5 µg, Imipenem (Ipm) -10 µg, Cefepimesulbactam (Cfs) – 75/10 µg, Amoxicillin-clavulanic acid (Amc) -20/10, Piperacillin-tazobactam (Pit) – 100/10 µg, Ceftriaxone / clavulanic acid (Cac) -30 /10 µg, Ceftazidime / clavulanic acid (Cfc) -30 /10 µg, Vancomycin -30 µg were obtained from Himedia Laboratories and used as per manufacturer's instructions.

High level gentamicin disc for used exclusively for testing *Enterococcus* species. Antibiotic susceptibility test for all bacterial isolates were performed based on Modified Kirby Bauer's disc diffusion method. Antibiotic discs were placed on the agar

plates and incubated overnight at 37°C for 24 hours. The zones of inhibition were measured and the isolates were classified as susceptible, intermediate, and resistant according to CLSI guidelines (CLSI, 2017). Quality control strains used for AST were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603.

Drug resistant gram negative bacilli producing extended spectrum beta lactamases (ESBL) and Methicillin resistance in Staphylococcal isolates (MRSA) were identified as per the methods of detection given below

ESBL screening test

As per the CLSI Screening criteria, the strain was considered to be “suspicious” of ESBL production if a zone diameter for the third generation cephalosporins was recorded as ≤ 22 mm for ceftazidime, and ≤ 25 mm for ceftriaxone. For *E. coli* and *Klebsiella* strains ceftriaxone zone ≤ 25 mm and ceftazidime zone diameter of ≤ 22 mm is considered to be resistant and indicates ESBL production.

ESBL Confirmatory Method

All the strains which were screened out for ESBL production were also subjected to confirmation by using the Phenotypic confirmatory disc diffusion test (CDDT), as recommended by the CLSI.

Discs of ceftazidime+ clavulanic acid (Cfc) - 30 µg /10 µg, and Ceftriaxone / clavulanic acid (Cac) -30 µg /10 µg, (Hi - media) were used as per CLSI guidelines. Organism was considered as ESBL producer if there was a more than or equal to 5 mm increase in zone diameter for ceftazidime and ceftriaxone tested in combination with clavulanic acid versus its zone when tested alone.

Detection of MRSA

Methicillin resistant *Staphylococcus aureus* strains were identified based on susceptibility to Cefoxitin 30 µg disc which is considered as surrogate marker for oxacillin used for detection of methicillin resistant isolates as per CLSI guidelines

Cefoxitin disc diffusion method

All strains were tested with 30 µg cefoxitin(Cx) discs (Hi-Media) on Mueller–Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. The zone of inhibition was determined after 16–18 hour incubation at 35°C. Zone size was interpreted according to CLSI criteria: susceptible, ≥ 22 mm; resistant, ≤ 21 mm. Those strains exhibiting zone diameter of ≤ 21 mm were considered as methicillin resistant.

Age, sex, clinical details of the patients included in this study were recorded in a proforma after obtaining informed consent from all the patients included in the study.

Results and Discussion

Total number of pus samples included in this study was 2455. Out of these 1836 (75%) samples showed growth of bacterial colonies and 619 (25%) samples did not show bacterial growth even after 48 hours of aerobic incubation as indicated in figure 1.

Socio demographic factors in patients with wound infections

In our study almost 1836(75%) of pus samples showed bacterial growth. 1303(71%) male patients were infected whereas only 533(29%) female patients were infected among the pus samples received. Common age group involved is 21–40 years.

Among 532 (22%) samples received from Accident and Emergency trauma ward, 459 (25%) of samples showed bacterial growth followed by 422 (23%), 294 (16%) and 257 (14%) from burns ward, postoperative ward, diabetology ward respectively as described in table 1.

Bacteria isolated were as follows

Klebsiella species was the most predominant bacterial pathogen 635 (35%) followed by *Staphylococcus aureus* 334 (18%), *Escherichia coli* 253 (14%), Coagulase negative *Staphylococcus* species 204 (11%), *Pseudomonas aeruginosa* 153 (8%), *Proteus* species 126 (7%), *Citrobacter* species 51 (3%), *Enterococcus* species, 43(2%) and *Acinetobacter* species 37 (2%) as described in figure 3.

Polymicrobial infection with two different organisms occurred in 22% of cases. Combined organisms were *Pseudomonas aeruginosa* with *Staphylococcus aureus* in 15% followed by *Klebsiella* species with Coagulase negative *Staphylococcus* species in 7% of isolates.

Extended spectrum beta lactamase (ESBL) producing gram negative bacterial isolates

ESBL producing isolates were found to be highest in *Klebsiella* species 311 (49%), *Escherichia coli* 124 (49%) followed by *Proteus* species 62 (49%), *Acinetobacter* species 18 (48%) and *Pseudomonas aeruginosa* 90 (59%). All the ESBL producing bacterial isolates were 100% sensitive to carbapenem group of drugs. Hence Amp C metallo-beta lactamase detection was not done. Screening test using Ceftriaxone, Ceftazidime disc was done and confirmation of the same was also done CDDT using Ceftriaxone clavulanic acid and

Ceftazidime clavulanic acid antibiotic disc as per guidelines.

ESBL production was detected in all the gram negative bacilli in varying percentage. Total gram negative bacilli producing ESBL was 626 (50%). Highest incidence was due to ESBL isolates of *Pseudomonas aeruginosa* 90 (59%) followed by *Klebsiella* sp 312(49%), *E. coli* 124 (49%), *Proteus* sp. 62(49%), *Acinetobacter* sp 18 (48%) and *Citrobacter* sp 18 (35%) as described in table 2 and figure 4.

Methicillin resistant Staphylococcal isolates

Methicillin resistance was detected by Cefoxitin disc diffusion method.

MRSA isolates were 96(43%) in *Staphylococcus aureus* isolates followed by 84(41%) in Coagulase negative Staphylococcal species. All the gram positive bacteria isolated were found to be sensitive to Vancomycin (100%). No vancomycin resistant strain was detected including *Enterococcus* species.

Klebsiella species isolated were 64% sensitive to piperacillin tazobactam, 63% sensitive to amikacin, 61% sensitive to tobramycin, 52% sensitive to ceftazidime, 51% to ceftriaxone, 49% sensitive to cefepime tazobactam and gemifloxacin, 48% sensitive to ceftriaxone.

Antibiotic resistance of *Klebsiella* species was higher to oral first, second and third generation cephalosporins and other beta lactam group of antibiotics.

E. coli isolates exhibited antibiotic susceptibility of 100% to imipenem, followed by 75% to gemifloxacin, 70% to amikacin, 63% to tobramycin, 63% to ciprofloxacin and 49% sensitivity to cefepime sulbactam combination.

Pseudomonas aeruginosa is 100% sensitive to Imipenem, 85% to tobramycin, 85% to Ciprofloxacin, 71% to netilmicin, followed by Piperacillin (51%), Ceftazidime (58%), cefepime sulbactam (59%), and piperacillin tazobactam (58%)

Gram positive bacteria mainly *S. aureus* exhibited 100% sensitivity to vancomycin and linezolid followed by 60% sensitivity to all beta lactam antibiotics and 59% to aminoglycosides.

All the ESBL producing gram negative bacilli isolated in our study showed 100% susceptibility to Carbapenems. All MRSA isolated showed 100% susceptibility to Vancomycin and Linezolid.

In our study almost 1836(75%) of pus samples showed bacterial growth. Male patients were more infected than female patients. Common age group involved is 21–40 years. This was similar to the study by Vijeta Sharma *et al.*, (2015).

Gram positive bacteria were isolated from 581 (32%) pus samples and gram negative bacteria were isolated from 1255 (68%) pus samples. Mixed growth was seen in 22% samples and no growth in 25% of pus samples.

Gram-negative bacteria were the dominant isolates from pus samples compared to Gram-positive bacteria which are in agreement to several earlier studies. This was similar to the studies by Rugira Trojan *et al.*, (2016), Bhatt and Vassikar (2010), Rajeshwar Rao *et al.*, (2014), Sarathbabu *et al.*, (2012), Kritu Panta *et al.*, (2013), Ravichitra (2014), Rajan (2014), Sankarankutty *et al.*, (2014) and Aizza Zafar (2008).

Bacterial pathogens were isolated from patients with the isolation rate of 75% similar to study by Dagnachew Muluye *et al.*, (2014)

This was higher than the results reported Mulu *et al.*, (2006) and Hima Bindu Mantravadi *et al.*, (2015).

Most infected wounds were due to trauma or injury, followed by postoperative infection of surgical wounds, diabetic foot ulcers followed by other minor lesions similar to the study by Ashby *et al.*, (2010).

Polymicrobial infection with two different organisms occurred in 22% of cases. Combined organisms were *Pseudomonas aeruginosa* with *Staphylococcus aureus* in 15% followed by *Klebsiella* species with Coagulase negative *Staphylococcus* species in 7% of isolates.

As per the study conducted by Lucinda J Bessa *et al.*, (2013), polymicrobial infection was found in 27% of the samples and was mainly constituted with two species. The most common association was *S. aureus/P. aeruginosa*. Similar to the association among organisms isolated in our study.

Klebsiella species was the most predominant organism followed by *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter* species similar to study by Vijeta Sharma *et al.*, (2015).

Most ESBL producers were isolated in *Klebsiella pneumoniae* and *Escherichia coli* and *Pseudomonas aeruginosa*. 43% of *Staphylococcus* species was methicillin resistant and all the isolates were sensitive to Vancomycin.

In this study highest occurrence were observed in male n%=1303(71%) as compared to female n (%) = 533 (29%). It was correlated with Siddqui *et al.*, (2002)

study which shows male preponderance (72%). Most commonly affected age group ranges between 21 – 40 years. This was similar to studies by Bhatt and Vassikar (2010) and Rajeshwar Rao *et al.*, (2014).

Klebsiella sp. was the most predominant organism 635 (35%). This study was similar to other authors like Sarathbabu *et al.*, (2012), Rajeshwar Rao *et al.*, (2014), Kritu Panta *et al.*, (2013) and Ravichitra (2014), Ananth and Rajan (2014) and Sankarankutty *et al.*, (2014).

Staphylococcus aureus was the next common organism 334 (8%) in the present study and correlated with other workers like Haitham *et al.*, (2011), Iman Hussain *et al.*, (2012). Aizza Zafar (2008) also has shown as second most disease causing organism.

However many workers have found most predominant organism in their studies like Farzana *et al.*, (2013), Ehmer Al-Ibram (2013) and Soumya Kaup and Jaya Sankarankutty (2014).

The present study reveals the incidence of infections due to *Klebsiella* species and their tendency towards antibiotic resistance. Multidrug resistant bacteria are emerging worldwide which causes major public health problems and challenges to health care.

Klebsiella species are 100 % sensitive to Imipenem, 63% of Amikacin, 52% Ceftazidime, 49% Gemifloxacin (which is considered as most preferred drug for treatment of *Klebsiella* species), 48% Cefotaxime,, 46% Ciprofloxacin, 22 % Tetracycline and 20% Amoxicillinclavulanic acid.

Table.1 Socio demographic factors in patients with wound infections

Demographic factors	Infected No. (%)	Not infected No.(%)	Total samples
Male	1303(71%)	611(99%)	1914(78%)
Female	533 (29%)	8 (1%)	541 (22%)
Total	1836	619	2455
Age in years			
10-20	184(10%)	39 (6%)	223 (9%)
21-30	587(32%)	69 (11%)	656 (27%)
31-40	454(25%)	39 (6%)	493 (20%)
41-50	220(12%)	273 (44%)	493 (20%)
51-60	204(11%)	190 (32%)	394(16%)
61-70	187(10%)	9(1%)	196 (8%)
Total	1836	619	2455
Wards and lesions			
Trauma ward	459 (25%)	81 (13%)	540 (22%)
Burns ward	422 (23%)	94 (15%)	516 (21%)
Postoperative wards	294 (16%)	123 (20%)	417 (17%)
Diabetic foot Ulcers	257 (14%)	111(18%)	368 (15%)
Abrasions, ulcers	202 (11%)	142 (23%)	344 (14%)
Others	202 (11%)	68 (11%)	270 (11%)
Total	1836	619	2455
Patients			
Inpatients	1791(98%)	580 (94%)	2371 (97%)
Outpatients	45(2%)	39 (6%)	84 (3%)
Total	1836	619	2455

Table.2 Gram negative bacilli producing ESBL

Bacteria isolated	ESBL isolates n (%)	Non ESBL isolatesn(%)	Total
<i>Klebsiella sp.</i>	312(49)	323(51)	635
<i>Escherichia coli</i>	124 (49)	129(51)	253
<i>Proteus sp.</i>	62(49)	64(51)	126
<i>Citrobacter sp</i>	18 (35)	33(65)	51
<i>Pseudomonas aeruginosa</i>	90(59)	63(41)	153
<i>Acinetobacter sp</i>	18(48)	19(52)	37
Total	624(50)	629(50)	1255

Table.3 Antibiotic susceptibility of gram negative bacteria to various groups of antibiotics

Organism	<i>Klebsiella sp</i>		<i>E.coli</i>		<i>Pseudomonas sp.</i>		<i>Proteus sp</i>		<i>Citrobacter sp</i>		<i>Acinetobacter sp</i>	
n (%)	635(35)		253(41)		153(8)		126(7)		51(3)		37(2)	
Antibiotics	S	R	S	R	S	R	S	R	S	R	S	R
AK	400(63)	235(37)	177(70)	76(30)	99(65)	54(35)	55(44)	71(56)	45(88)	6(12)	34(92)	3(8)
Gen	362(57)	273(43)	146(58)	107(42)	66(43)	87(57)	35(28)	91(72)	43(85)	8(15)	27(72)	10(28)
Tob	387(61)	248(39)	159(63)	94(37)	130(85)	23(15)	52(41)	74(59)	48(94)	3(6)	34(92)	3(8)
Net	375(59)	260(41)	167(66)	86(34)	109(71)	44(29)	53(42)	73(58)	46(97)	5(3)	31(84)	6(16)
Amp	133(21)	502(79)	116(46)	137(54)	37(24)	116(76)	60(48)	66(52)	15(29)	36(71)	10(28)	27(72)
Pi	133(21)	502(79)	124(49)	129(51)	78(51)	75(49)	68(54)	58(46)	21(41)	30(59)	15(40)	22(60)
Cz	133(21)	502(79)	127(50)	127(50)	0	0	64(51)	62(49)	22(44)	29(56)	0	0
Cn	127(20)	508(80)	116(46)	137(54)	0	0	60(48)	66(52)	22(44)	29(56)	0	0
Cxm	140(22)	495(78)	134(53)	119(47)	0	0	57(54)	69(46)	32(62)	19(38)	0	0
Cfm	133(21)	502(79)	142(56)	111(44)	0	0	67(53)	59(47)	33(65)	18(35)	0	0
Ctr	323(51)	311(49)	129(51)	124(49)	113(74)	40(26)	64(51)	62(49)	33(65)	18(35)	19(52)	18(48)
Cpz	311(49)	323(51)	124(49)	129(51)	63(41)	57(37)	73(58)	53(42)	36(71)	15(29)	18(48)	19(52)
Caz	331(52)	305(48)	127(50)	126(50)	63(41)	90(59)	68(54)	38(46)	29(56)	22(44)	19(52)	18(48)
Ctx	305(48)	331(52)	132(52)	121(48)	96(63)	57(37)	64(51)	62(49)	29(56)	22(44)	18(48)	19(52)
Clr	133(21)	502(79)	116(46)	137(54)	66(43)	87(57)	49(39)	77(61)	22(44)	29(56)	15(40)	22(60)
Do	140(22)	495(78)	94(37)	159(63)	44(29)	109(71)	45(36)	81(64)	18(35)	33(65)	15(40)	22(60)
Cip	292(46)	343(54)	91(63)	162(37)	130(85)	23(15)	40(32)	86(68)	29(56)	22(44)	30(80)	7(20)
Gem	311(49)	323(51)	190(75)	63(25)	121(79)	32(21)	64(51)	62(49)	33(65)	18(35)	33(88)	4(12)
Of	273(43)	362(57)	127(50)	126(50)	109(71)	44(29)	34(27)	92(73)	18(35)	33(65)	18(48)	19(52)
Cot	133(21)	502(79)	119(47)	134(53)	0	0	35(28)	91(72)	19(38)	32(62)	0	0
Cfs	311(49)	323(51)	124(49)	129(51)	90(59)	63(41)	66(52)	60(51)	18(35)	33(65)	16(44)	21(56)
Amc	127(20)	508(80)	139(55)	114(45)	47(31)	106(71)	34(27)	92(73)	16(32)	35(68)	9(24)	28(76)
Pit	406(64)	229(36)	134(53)	119(47)	89(58)	64(42)	72(57)	54(43)	22(44)	29(56)	33(88)	4(12)
Ipm	635(100)	0(0)	253(100)	0(0)	152(100)	0(0)	126(100)	0(0)	51(100)	0(0)	37(100)	0(0)

Figure 1 - Percentage of samples with bacterial growth

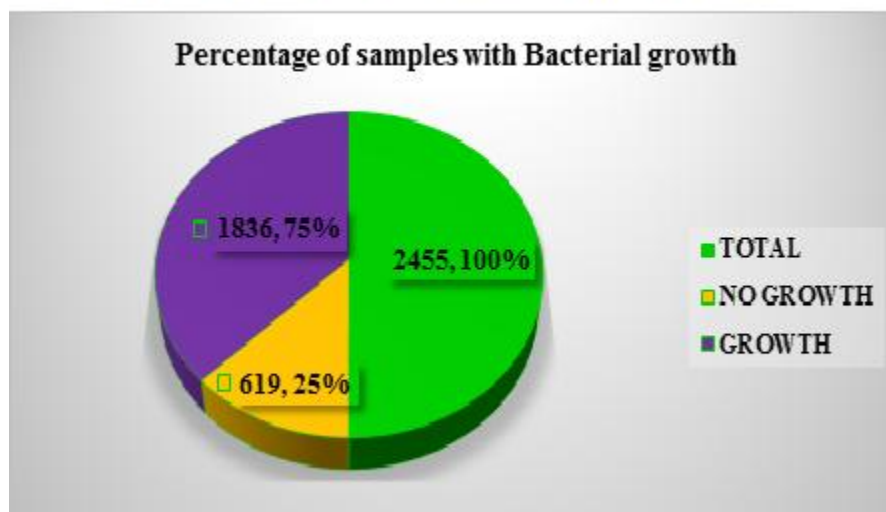
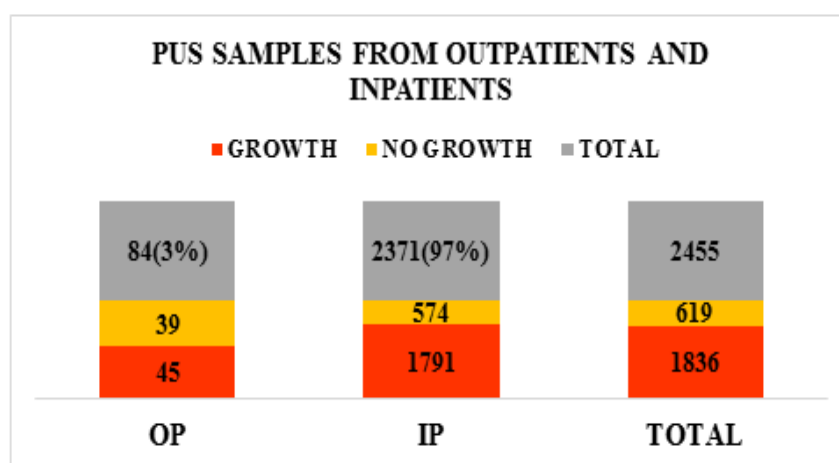


Table.4 Antibiotic susceptibility of gram positive bacteria to various groups of antibiotics

Organisms	<i>S.aureus</i>		CONS		<i>Enterococci</i>	
	n(%)	334(18)	204(11)		43(2)	
Antibiotics	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Amp	194(58)	140(42)	122(60)	82(40)	14(33)	29(67)
Pi	197(59)	137(41)	120(59)	84(41)	29(67)	14(33)
AK	197(59)	91(41)	139(68)	53(32)	0	0
Gen (HLG)	160(48)	115(52)	114(56)	90(44)	43(100)	0(0)
Cx	190(57)	96(43)	120(59)	84(41)	0	0
Clr	73(22)	173(78)	39(19)	165(81)	0	0
Azm	70(21)	174(79)	37(18)	167(82)	0	0
Do	90(27)	163(73)	55(27)	149(13)	14(33)	29(67)
Cot	67(20)	178(80)	65(32)	139(68)	0	0
Cip	47(14)	192(86)	75(37)	129(63)	14(33)	29(67)
Gem	43(13)	194(87)	96(43)	108(57)	29(67)	14(33)
Of	43(13)	195(87)	63(31)	141(69)	14(33)	29(67)
Cn	204(61)	130(39)	122(60)	82(40)	0	0
Cxm	200(60)	134(40)	124(61)	80(39)	0	0
Ctr	190(57)	144(43)	120(59)	84(41)	0	0
Cfs	190(57)	144(43)	118(58)	86(42)	0	0
Amc	197(59)	137(41)	120(59)	84(41)	29(67)	14(33)
Pit	194(58)	140(42)	116(57)	88(43)	43(100)	0(0)
Van	334(100)	0(0)	204(100)	0	43(100)	0(0)
Lz	334(100)	0(0)	204(100)	0	43(100)	0(0)

Figure 2 - Pus samples from OP and IP



Gram positive bacteria were isolated from 581 (32%) pus samples and gram negative bacteria were isolated from 1255 (68%) pus samples. 73% males and 27% females showed bacterial growth. Out of the total samples bacteria was isolated from 84 (3%) outpatients and 2371(97%) inpatients from various wards of our hospital as in Figure 2

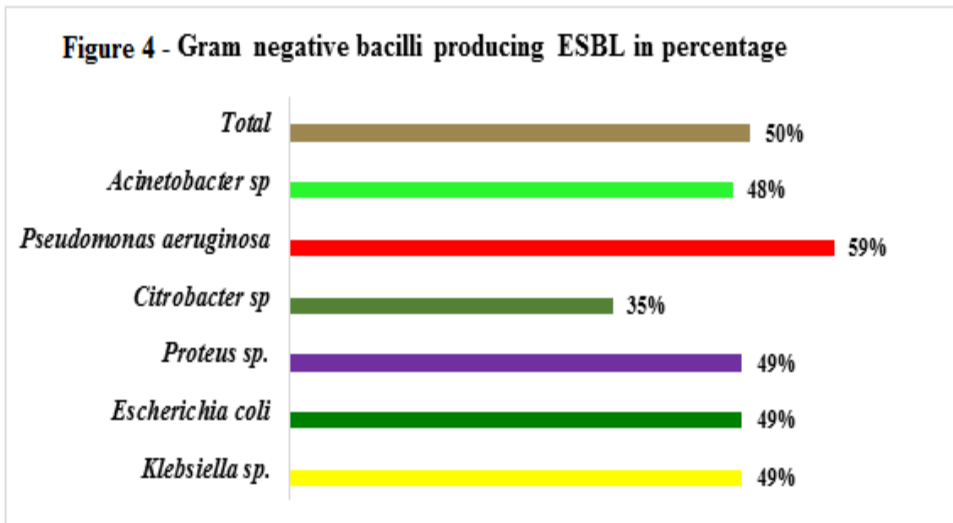
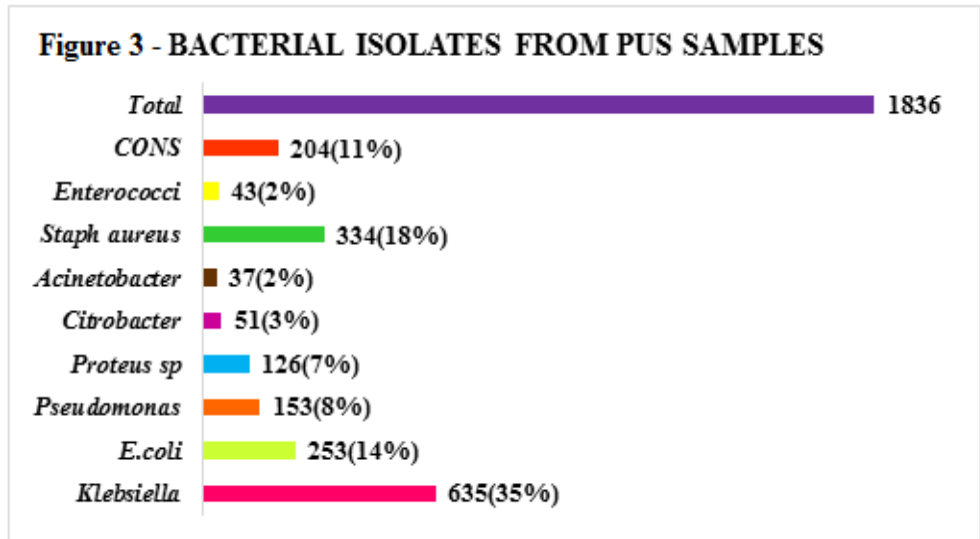


Figure.5 Phenotypic confirmatory double disc diffusion test for ESBL isolates



Figure 6

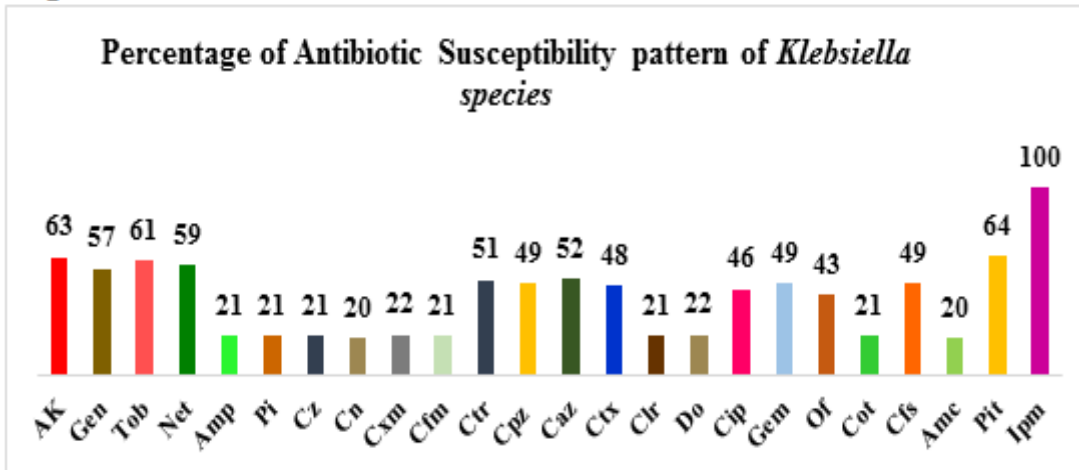
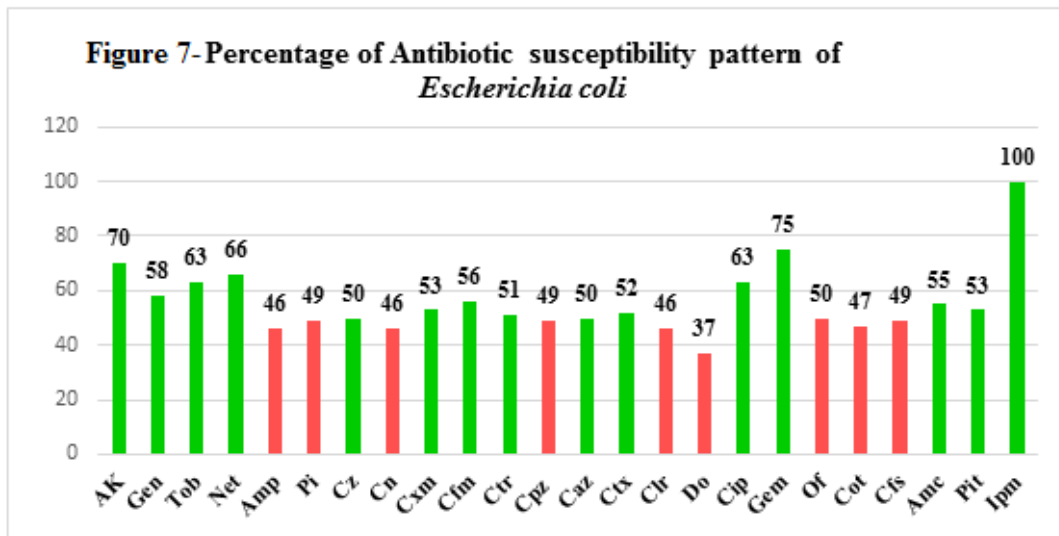
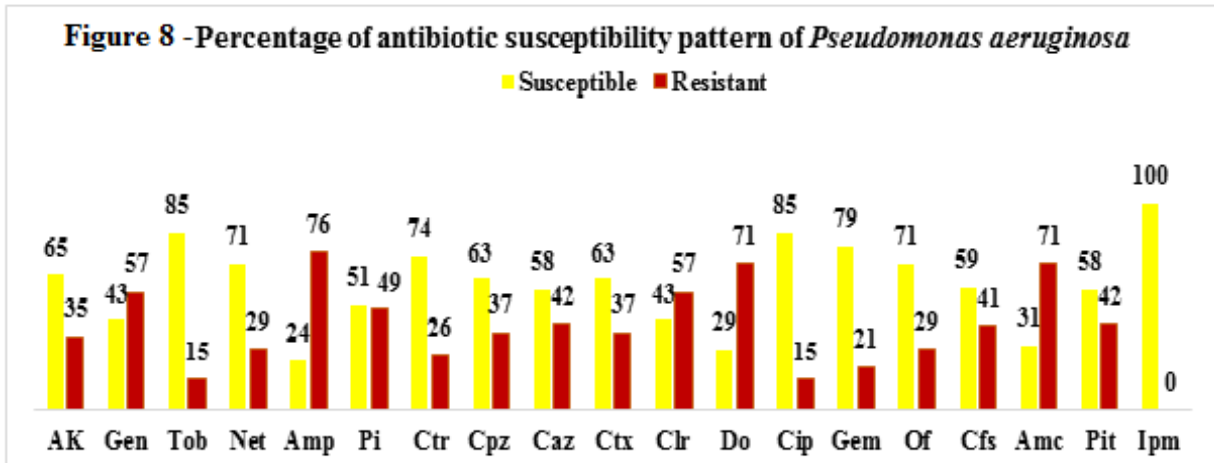


Figure 7-Percentage of Antibiotic susceptibility pattern of *Escherichia coli*



In the above figure all the *E.coli* isolates with sensitivity more than 50% is highlighted in green and those with <= 50% sensitivity is in red colour.

Figure 8 -Percentage of antibiotic susceptibility pattern of *Pseudomonas aeruginosa*



As most of the empirically preferred antibiotics are almost susceptible to gram negative bacilli the use of the same as above shall be implemented for treatment of *Klebsiella* infections. This finding is similar to correlate with Zhang *et al.*, (2014) who reported predominance of *K. pneumoniae*, *E. coli*, *S. aureus* and *P. aeruginosa* in pus samples from patients with severe wound infection.

E. coli isolates exhibited antibiotic susceptibility of 100% to imipenem, followed by 75% to gemifloxacin, 70% to amikacin, 63% to tobramycin, 63% to ciprofloxacin and 49% sensitivity to cefaperazonesulbactam combination.

Pseudomonas aeruginosa is 100% sensitive to Imipenem, 85% to tobramycin, 85% to Ciprofloxacin, 71% to netilmicin, followed by Piperacillin (51%), Ceftazidime (58%), cefaperazonesulbactam (59%), and piperacillintazobactam (58%).

These studies were compared to other studies like Soumya Kaup and Jaya Sankarankutty (2014), Rajeshwaretal (2014) as these showed similarities in antibiotic sensitivity pattern.

Gram positive bacteria mainly *S. aureus* exhibited 100% sensitivity to vancomycin and linezolid followed by 70% sensitivity to macrolide group of antibiotics, 60% sensitivity to all beta lactam antibiotics and 59% to aminoglycosides.

Multidrug resistance (Resistant to one or more antibiotic classes) was found to be predominant in *Klebsiella pneumoniae* followed by *Staphylococcus aureus*. Hence indiscriminate use of antibiotics and irregular treatment shall be avoided as this study has expressed the percentage of bacterial isolates susceptible to all antibiotics of common use. The spread of ESBL-producing bacteria has

been strikingly rapid worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required as per the study by Basavaraj C. Metri *et al.*, (2011).

The cefoxitin disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute, was found to be a reliable method for MRSA detection Priya Datta *et al.*, (2011). Incidence of 35%- 40% MRSA was detected in study by Raghavendra Adhikari *et al.*, (2017) and Rashmi *et al.*, (2017). Mohammad Fareed Khan *et al.*, (2015) also has detected that 35% of all *S. aureus* infections had been caused by MRSA.

In this study ESBL isolates has been detected by different gram negative bacilli with high incidence in *Klebsiella* sp followed by *Escherichia coli*, *Proteus* sp, *Citrobacter* species among gram negative bacilli under family Enterobacteriaceae. Among non-fermenting gram negative bacilli high incidence of ESBL was detected in *Pseudomonas aeruginosa* followed by *Acinetobacter* species.

All MRSA isolates were sensitive to vancomycin, linezolid, mupirocin and rifampicin similar to the study by Arunava Kali *et al.*, (2013). Verma *et al.*, has reported a rapid increase in MRSA prevalence, from 12% to 80.89%, over seven years, in a tertiary care centre at Indore. As per study by Srikanth *et al.*, (2013), 40% among *Staphylococcus* species were methicillin resistant. This is in similarity to our study.

In contrast, very low incidence of 26% and high incidence of 78% had been reported by Pandey *et al.*, (2012) and Masoumeh Navidinia *et al.*, (2015).

This variation may be due to other associated factors like less adherence to hygienic

practices, sample load of patients where more possibility for transmission of MRSA shall be considered to be the main cause.

ESBL production was detected in all the gram negative bacilli in varying percentage. Total gram negative bacilli producing ESBL was 626 (50%). Highest incidence was due to ESBL isolates of *Pseudomonas aeruginosa* 90 (59%) followed by *Klebsiella* sp 312(49%), *E. coli* 124 (49%), *Proteus* sp. 62(49%), *Acinetobacter* sp 18 (48%) and *Citrobacter* sp 18 (35%).

Klebsiella pneumoniae was the most common ESBL producing Enterobacteriaceae, followed by *E. coli* and others, as per Basavaraj C. Metri *et al.*, (2011) which is similar to this study. Nazneen Siddiqui *et al.*, 2014 has reported 61% were found to be ESBL positive by the CDDT method. The different gram negative bacilli identified were *Klebsiella pneumoniae* (47%). This is also similar to figures reported in our study.

ESBL production was most common in *Klebsiella pneumoniae* (74%), followed by *E. coli* (62%) as per Ankur Goyal *et al.*, (2009). This was found to be higher than that reported in our study. Imipenem showed the highest rate of sensitivity (97%) almost similar to our study with 100% sensitivity to imipenem.

Baby Padmini *et al.*, have shown 40% and 41% ESBL positivity among *K. pneumoniae* and *E. coli* respectively and Vinod Kumar *et al.*, from Gulbarga reported 16.8% and 48.6% of *E. coli* and *K. pneumoniae* respectively as the ESBL producers. This is similar to finding in our study. Basavaraj *et al.*, (2011).

As per Ankur Goyal *et al.*, (2009), occurrence of ESBL was reported to be 63.6 % in *E. coli* and 66.7 % in *K. pneumoniae*. This figure is higher than that reported in our study which may be due to variable distribution of normal

microbial flora and indiscriminate use of antibiotics leading to increasing multi drug resistance.

As per the study by Shaoyi Zhang *et al.*, (2014) majority of patients admitted with wound infections were due to trauma or injury and among those who were admitted to the emergency room immediately following trauma, majority of cases were complicated with shock. High degree of resistance was observed among gram negative organisms to beta lactam groups of antibiotics. The percentage of oxacillin resistance among staphylococcal isolates was 33.1%, but all were sensitive to vancomycin as per the study by Iff at Javeed *et al.*, (2011) and these correlate with our findings in this study.

No bacterial isolates was found to be sensitive to all antibiotics tested. Isolated bacteria showed multi drug resistance to the commonly used antibiotics in the hospital.

Most of the Gram negative bacteria and Gram positive isolates were sensitive to Gentamicin and Vancomycin, respectively as per the study by Mengesh *et al.*, (2014) which is similar to this study

As most of the figures in various studies conducted since years were similar to the findings in our study the rate of incidence of bacterial isolates with their antibiotic susceptibility pattern, sociodemographic factors related to wound infections have been widely described.

Similarly, all the relevant information about the bacterial pathogens isolated from the pus samples and antibiotic guideline to be followed by all the clinicians for empirical treatment of the patients in case of accidental trauma, other emergencies and for outpatients has been outlined in this study.

This would be the baseline for formulation of policies for prevention of emerging multi drug resistant bacterial isolates in our hospital. In addition to this proper hygiene and sanitation measures shall be adopted by all the health care personnel of our hospital among which adoption of hand hygiene which would be considered to be of prime importance.

Most important points to be considered includes negligence on patients part, incomplete treatment schedules, antibiotic misuse, self-prescription, misprescription, lack of regional antibiogram data, and limited knowledge about multidrug-resistant isolates and antimicrobial resistance among clinicians.

Routine review and updates of antimicrobial susceptibility profiles of clinical isolates will help in formulating the most appropriate antibiotic to be prescribed in our hospital, dose-regimen and treatment schedule against wound infections but also help in decreasing the emerging and alarming drug resistant bacterial isolates.

All the patients received in our hospital has to be made aware of the need for hygiene and sanitation measures which shall be an insight to the society for prevention of emerging multidrug resistant isolates and spread of the same.

In conclusion, wound infections are more prevalent due to lack of awareness about hygienic practices, empirical treatment of patients with irrelevant antibiotics use.

This study was carried out in our facility to provide relevant information with regard to bacteria causing wound infections.

Antibiotic susceptibility to various group of antibiotics were interpreted based on CLSI guidelines which has outlined the basic idea

for antibiotics that shall be prescribed in our facility empirically.

As emerging antibiotic resistance of bacterial isolates has posed a threat to the entire society this study has enlightened the necessity of proper selection of antibiotics and to administer the appropriate dosage and duration of their use as per the pharmacological protocols. Hence strict adherence to formulated antibiotic policies and infection control measures suitable for our set up has to be considered essential.

This study has also defined the socio demographic factors in epidemiology of wound infections. Incidence of wound infections and multidrug resistant organisms has also been derived. As this type of prospective study has not been conducted so far in our hospital this data would be helpful to cater the needs of the patients with wound infections.

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