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Antibiotics Sensitivity Pattern of Post-Operative Wound Infections in a Tertiary Care Hospital, Western Nepal

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Abstract

Background: Surgical site infections (SSIs) is one of the most common postoperative complications and cause significant postoperative morbidity, mortality, prolong hospital stay and increase in hospital cost. The condition is serious in developing countries like Nepal owing to irrational prescriptions of antimicrobial agent. SSIs in those countries rates from 2.5% to 41.9%. This study was performed to find the common organisms causing surgical site infections and their antibiotics sensitivity pattern in a tertiary care hospital, western Nepal.

Materials and methods: Pus or swab samples collected from suspected post- operative wound infections and submitted for culture and sensitivity in the Department of Microbiology were included in this study. Isolation and identification of the organisms was done as recommended by American society of microbiology (ASM). Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI) guideline.

Results: Out of 152 pus and swab samples processed for culture, (64.5%) showed culture positivity. In total isolates (65.7%) were Gram negative bacteria and (34.3%) Gram positive bacteria. *Staphylococcus aureus* (23.9%) was the predominant Gram positive isolate and *Escherichia coli* (18%) was the major Gram negative isolate. *S. aureus* showed (100%) sensitivity towards Linezolid and (94.4%) towards Vancomycin. Among commonly used antibiotics for Gram positive bacteria Penicillin (94.4%), Erythromycin (80.5%) were highly resistant. Sixty percent of S*taphylococcus aureus* isolated showed methicillin resistant (MRSA). Gram negative bacteria showed (100%) sensitivity towards the Colistin sulphate and Polymyxin B and were highly resistant towards Ampicillin (98.2%), Cefexime (87.3%), Ceftriaxone (87.3%) and other commonly used antibiotics. Overall multi-drug resistance was found in (89.5%) isolates. Among Gram negative bacterial isolates (23.1%) were MBL producer and (21.7%) were ESBL producer.

Conclusion: Culture positivity in suspected case of SSIs was high (65.1%). *Staphylococcus aureus* was the common causative agent of SSIs. Bacteria showed more than 50% resistance towards commonly used antibiotics. So for the selection of appropriate antibiotic for better treatment of patients, culture and sensitivity should be done in every suspected case of SSIs.

1. Background

Surgical site infections (SSIs) is one of the most common postoperative complications and cause significant postoperative morbidity, mortality, prolong hospital stay and increase in hospital cost [1]. The United state centre's for disease control and prevention (CDC) has developed criteria that define surgical site infections as infections related to an operative procedure that occur at or near the surgical incision within 30 days or within 1 year if prosthetic material is implanted at surgery. SSIs are a real problem to the surgeons and are considered as major infection control concern across the world [2].

Unrestrained and rapidly spreading anti-microbial resistance among bacterial population become a serious challenge in the management and treatment of patients with post operative wound infections. Most post operative wound infections are hospital acquired and vary from one hospital to the other hospital, one surgeon to another surgeons, one patient to another patient and are associated with complication of increased morbidity and mortality [3, 4]. The emergence of antimicrobial resistance has made the choice of empirical therapy more difficult and expensive [5]. The condition is serious in developing countries due to irrational prescriptions of antimicrobial agent [6]. SSIs in those countries rates from 2.5–41.9% [7]. This study was conducted to assess the current status of bacterial pathogen involved in post operative wound infections and their antibiotics sensitivity test (AST) pattern in Universal college of Medical sciences and teaching hospital (UCMS-TH), Bhairahawa, Nepal.

Methods

This observational and cross-sectional study was conducted at Department of Microbiology in collaboration with Department of Surgery at Universal College of Medical Sciences (UCMS), over a period of six months, March to September 2019. A total of 152 pus or swab samples collected from patients suspected of surgical site infection were studied.

The methods for the collection, isolation, and identification were performed as described by American Society of Microbiology (ASM) and analyzed accordingly [8]. Collected pus or swab samples from suspected infected site were inoculated on Blood agar (BA) and MacConkey agar (MAC) (HiMedia) plates. The plates were incubated at 37 °C for 24 hr. All isolated colonies growing in the BA and MAC agar were processed further for identification.Patients of all age groups with suspected post operative SSIs admitted in different wards with their written consent were enrolled for the study.Patients were excluded from the study if they had wound infection other than postoperative wound, Infection occurring 30 days after operation if there is no implant and after 90 days if implant is in place, Burn injuries and donor sites of its skin grafts. The specimen not fulfilling the criteria of ASM was also excluded from the study.

Identification of Bacterial Isolates

Identification of the isolates were done by the following standard microbiological techniques which involved morphological appearance of the colonies, Gram's staining reactions, catalase test, oxidase test, and other biochemical properties, for example, Sulphide Indole Motility (SIM) media, Simmons citrate media, Christensen's urea agar, Triple Sugar Iron agar (TSI), Decarboxylase test media, Hugh and Leifson's OF (oxidative and fermentative) test media, MR/VP (methyl red/Voges Proskauer) broth, Phenylalanine agar, Nitrate reduction test, and others as required [9] .

Phenotype Detection for ESBL

The initial screening test for the production of ESBL was performed by using ceftazidime (CAZ) (30 *μg*) and cefotaxime (CTX) (30 *μg*) disks (Hi.Media India.). If the zone of inhibition (ZOI) was ≤ 22 mm for ceftazidime and ≤ 27 mm for cefotaxime, the isolate was considered as a potential ESBL producer. The

organism was swabbed on to a MHA (Mueller-Hinton agar) plate as done for the screening test in the antibiotic sensitivity test. Then, the combination disk method (CD) was applied for the confirmation of ESBL-producing strains [10].

Double disk synergy test

Amoxycillin- clavulanic acid (AMC) disk (20/10 µg) was placed at the center and disks containing the 30 µg of CAZ, CTX and CRO were placed separately beside 15 mm distance (edge to edge), away from the central disk, in a horizontal manner. Any enhancement of the zone of inhibition between the disks (either of the cephalosporin disks and clavulanic acid containing disk) indicated the presence of ESBL. Isolates with such pattern were recorded as ESBL producers [10].

Combination Disk (CD) Method

CD methods were used for the confirmation of ESBL-producing strains in which CAZ and CTX alone and in combination with clavulanic acid (CA) (10 µg) were used. An increased ZOI of ≥ 5 mm for either antimicrobial agent in combination with CA versus its zone when tested alone confirmed ESBL [10]. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative controls, respectively.

Tests for MBL Production

Screening test

The isolates were subjected for MBL detection when the ZOI for CAZ (30 µg) was < 18 mm [11].

MBL confirmation by combination disk (CD) method

Two imipenem (IPM) disks (10 µg) were used. In one of them, 10 µL of 0.1 mol/L (292 µg) anhydrous ethylenediaminetetraacetic acid (EDTA) was added. Then the two disks were placed 25 mm apart (center to center). An increase in zone diameter of > 4 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for an MBL [10].

Tests for MRSA

30 µg of cefoxitin disk method as recommended by CLSI was put up and agar plates were incubated at 35˚C. The diameter of the zone of inhibition of growth were recorded and interpreted as susceptible or resistant by the criteria of CLSI. *S aureus* strains ATCC 25923 and ATCC 43300 were used as negative and positive controls respectively. Organisms were considered methicillin resistant when the zone of inhibition was equal or less than 21 mm for *S. aureus* with cefoxitin disk method [10]

Antibiotic Susceptibility Testing

The antimicrobial susceptibility tests were performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (HiMedia, India) as per CLSI recommendations [10]. The antibiotics tested in this

study include amoxicillin (10 *µg*), ceftazidime (30 *µg*), cefotaxime (30 *µg*), cefoxitin (30 *µg*), cefepime (30 *µg*), aztreonam (30 *µg*), amoxicillin-clavulanate (30 *µg*), piperacillin-tazobactam (100/10 *µg*), gentamicin (10 *µg*), imipenem (10 *µg*), ciprofloxacin (5 *µg*), and cotrimoxazole (25 *µg*), respectively. All the antibiotics used were purchased from HiMedia Laboratories, Mumbai, India. Interpretation of antibiotic susceptibility results was made according to standard interpretative zone diameters suggested in CLSI guidelines [12]. In this study, if the isolates were resistant to at least three classes of first-line antimicrobial agents, they were regarded as MDR (multidrug resistance) [13].

Data Processing and analysis

All the data from cases were entered in MS Excel (Microsoft office 2007) and then analyzed by statistical package for social sciences (SPSS) for window version; SPSS 20 Inc, Chicago IL) All the data were expressed in the term of percentage frequency, mean ± SD and compared by Chi-square test. P-value < 0.05 was considered to be statistically significant.

Ethical Consideration

Study was approved by Institutional Review committee of Universal College of Medical Sciences, UCMS. Written informed consent was obtained from each individual participating in the study.

Results

A total of 152 specimens representing surgical site infection from different wards were processed and significant bacterial growth was found in 99 (64.5%) specimens. Among 99 specimens with significant growth, mixed bacterial growth was observed in 5 (5%) samples and monobacterial growth was found in 94 (95%) samples. Total number of bacteria isolated was 105. Out of 105 bacteria isolated from 99 samples, 69 (65.7%) were Gram negative and 36 (34.3%) were Gram positive bacterial isolates.

Staphylococcus aureus 25 (23.9%) was most commonly isolated organism followed by *Escherichia coli* 19 (18%), *Acinetobacter* species 16 (15.2%) and others. The details of organism profile are elicitated in Fig. 1.

Antimicrobial susceptibility test showed variable degree of resistance [Table 1, 2]. Ninety-four percentages of Gram positive bacterial isolates were resistant to penicillin, (80.6%) to Erythromycin and (68%) to cefoxitin. Regarding gram-negative bacteria, (98.2%) of the isolates were resistant to ampicillin, (87.3%) to ceftriaxone, cefexime and 80% to ceftriaxone. MDR isolates accounted for (89.5%) of the 105 isolates. Sixty percent of *Staphylococcus aureus* were methicillin resistant (MRSA). Among Gram negative isolates, (23.1%) were ESBL producers and (21.7%) were MBL producers. (Fig. 2) The rate of MDR was highest in *Citrobacter freundii* (100%) followed by *Acinetobacter* spesies (93.75%). Among 16 ESBL producing organisms, maximum isolates were *Proteus mirabilis* (50%) followed by *Escherichia coli* (31.6%) and *Pseudomonas aeruginosa* (28.6%). Maximum MBL producing bacteria was Acinetobacter species (37.5%) followed by *Klebsiella pneumoniae* (33.3%). (Fig. 2. Table 3)

Table 1
Antibiotics sensitivity pattern in Gram positive bacteria

Antibiotics	No of isolates tested	Sensitivity pattern			
		Sensitive		Resistant	
		No.	%	No.	%
Penicillin	36	2	5.6	34	94.4
Cefoxitin	32	10	31.3	22	68.7
Vancomycin	**36**	**34**	**94.4**	**2**	**5.6**
Erythromycin	36	7	19.4	29	80.6
Gentamicin	36	25	69.4	11	30.6
Tobramycin	36	30	83.3	6	16.7
Ceftriaxone	33	13	39.4	20	60.6
Cotrimoxazole	33	21	63.6	12	36.4
Cefexime	33	11	33.3	22	66.7
Cloxacillin	32	14	43.8	18	56.2
Cefepime	33	12	36.4	21	63.6
Doxycycline	32	29	90.6	3	9.4
Clindamycin	33	26	78.8	7	21.2
Linezolid	**32**	**32**	**100**	**0**	**0**

Table 2
Antibiotics sensitivity pattern in Gram negative bacteria (N = 69)

Antibiotics	Total isolates tested	Sensitivity pattern			
		Sensitive		Resistant	
	No	No.	%	No.	%
Ampicillin	55	1	1.8	54	98.2
Gentamicin	69	36	52.2	33	47.8
Tobramycin	69	40	57.9	29	42.1
Ciprofloxacin	55	11	20	44	80
Cotrimoxazole	55	18	32.7	37	67.3
Cefexime	55	7	12.7	47	87.3
Cefepime	55	12	21.8	43	78.2
Ceftriaxone	55	7	12.7	47	87.3
Piperacillin + Tazobactam	69	30	43.5	39	56.5
Meropenem	69	32	46.4	38	53.6
Levofloxacin	69	33	47.8	36	52.2
Doxycycline	55	30	55.6	24	44.4
Polymyxin B	**69**	**69**	**100**	**0**	**0**
Colistin sulphate	**69**	**69**	**100**	**0**	**0**

Table 3
Pattern of Gram negative isolates

S.N	Organism	Significant growth		MDR	ESBL	MBL
		NO.	%	No. (%)	No. (%)	No. (%)
1	*E.coli*	19	27.5	18 (94.7)	6 (31.6)	3 (15.8)
2	*Acinetobacter* species	16	23.2	15 (93.8)	2 (12.5)	6 (37.5)
3	*Klebsiella pneumonae*	15	21.7	14 (93.3)	3 (20)	5 (33.3)
4	*Pseudomonas aeruginosa*	14	20.3	12(64.3)	3 (28.6)	1 (7.1)
5	*Citrobacter freundii*	3	4.4	3 (100)	0 (0)	1 (33.3)
6	*Proteus mirabilis*	2	2.9	1 (50)	1 (50)	0 (0)
Total		**69**	**100**	**60 (86.9)**	**15 (21.7)**	**16 (23.2)**

Discussion

Out of 152 samples 99 (65.1%) showed culture positivity which is in accordance with the study done by khyati J et al. which showed a culture positivity of (62%) samples [14]. The study done by chaudhary et al. **s**howed culture positivity of (77.6%) which was higher than our study and another study done by Bastola et al. showed (48.6%) culture positivity which was lower than our study [15, 16]. Contamination from the external environment and poor hospital hygiene may be the possible reason for higher rate of surgical site infection [17].

In our study, (95%) of culture positive samples reveled mono-microbial growth and (5%) showed poly-microbial growth. Similarly high percentage (91.6%) of mono-microbial growth was reported by Mama M et al. in the year 2014 [18]. Similarly Acharya J et al. showed (10.5%) mixed bacterial growth [19].In total isolated organisms growth of Gram negative bacteria was (65.7%) which was more than growth of Gram positive bacteria (34.3%), It was similar with study done by Dhakal et al. in the year 2017 [20]. But in contrast Chaudhary et al. showed Gram positive bacteria were more common than Gram negative bactaeria [15]. This variation could be due to the diversity in study population, site of surgery and hospital environment [21].

Although Gram negative bacteria were more than Gram positive bacteria, common causative agent of SSIs in this study was *Staphylococcus aureus* (23.9%) which was in accordance with the study done by Dhakal et al., Chaudhary et al. and Sawadekar et al. [20, 15, 22]. *E.coli* was the most common Gram negative bacteria followed by Acinetobacter species and *Klebsiella pneumoniae* in our study. Similar study done by Nishanthy M. and Chitralekha et al. showed *Klebsiella* species was the most common Gram negative bacteria followed by *Pseudomonas aeruginosa, Escherichia coli, Proteus species* and *Acinetobacter* [23].The high prevalence of *S. aureus* infection may be because it is an endogenous source of infections. Infections with this organism may also be due to contamination from the environment e.g.

contamination of surgical instruments. With the disruption of natural skin barrier *S. aureus*, which is a common bacterium on surfaces, easily find their way into wounds .Gram negative bacteria were also encountered in high percentage this may be due to contamination of surgical wounds with normal flora of gut [24].

Resistance to the selected antimicrobials was very high. The overall multiple drug resistance of the isolates in this study was (89.5%) in total and (86.9%) among GPC and (91.7%) among GNB, which was in line with study done by Mulu W et al. and Mama M et al [25, 18]. But the study done by Pirvanescu H et al. encountered only (27.6%) MDR [26]. High resistance of the isolates to antibiotics may be due to practicing self medication or unavailability of guideline regarding the selection of drugs thereby which lead to inappropriate use of antibiotics [6].

Methicillin resistant *Staphylococccus aureus* was (60%) in the present study and was similar to the study by Bastola et al. who encountered (58.3%) MRSA [16]. Similarly the rate of MRSA was 48.78% in a study conducted by Jain et al. in India [27].

In isolated Gram positive bacteria Vancomycin and Linezolid were most effective antibiotics and other effective drugs were Doxycycline (90.6%), Tobramycin (83.3%) and Gentamicin (69.4%). In the other hand Penicillin (94.4%), Erythromycin (80.6%) and Cefexime (66.7%) were highly resistant. In similar study done by Raja et al. reported Vancomycin was the most effective antibiotic against the gram positive bacteria [28]. Aminoglycosides were effective against both Gram positive and Gram negative bacteria. Dhakal et al. in the year 2017 showed most effective antibiotics for Gram positive bacterial isolates was Clindamycin (71.4%) followed by Gentamicin (64.5%) while Ciprofloxacin (31.2%) was found to be the least effective antibiotic in GPC [20]. Study done by Nishanthy M and Chitralekha S in the year 2016 showed that most sensitive antibiotcs were Penicillin, Cefoxitin, Linozolid, Vancomycin and Cotrimoxazole in GPC [23].

Among the isolated Gram negative bacteria Colistin sulphate and Polymyxin B were 100% sensitive and other effective antibiotics were Tobramycin (57.9%), Doxycycline (55.6%), and Gentamicin (52.5%). Ampicillin was 100% Resistant and commonly used antibiotics Ceftriaxone, Ciprofloxacin; Cotrimoxazole also encountered resistant which is matter of concern. Similar study done by Kokate et al. showed GNB were maximum sensitivity towards Piperacillin-tazobactam, Imepenem and Polymyxin B [29]. Dhakal et al. showed that most of the Gram negative bacterial isolates were found to be sensitive to Amikacin (87.2%) followed by Gentamicin (67.9%) and Ceftriaxone (54.5%). Ampicillin (16.1%) was the least effective antibiotic among Gram negative bacterial isolates [20].Nepal is a developing country there is no specific rules for the purchase and use of antibiotics. People can use antibiotics without prescription. Irrational use of antibiotics is a major cause for drug resistant. Our study included indoor patients who get infection with already drug resistant bacteria from hospital source this may be the reason why most routinely used antibiotics were resistant [30, 31, 32].

In total Gram negative bacteria ESBL producing bacteria was (21.7%). *Proteus mirabilis* (50%) was common ESBL producing bacteria followed by *E.coli* (31.6%), *Pseudomonas aeruginosa* (28%), *Klebsiella*

pneumoniae (20%) and *Acinetobacter* (12.5%). MBL producing bacteria were (23.1%) in which Acinetobacter *species* (37.5%) was major MBL producer followed by *Klebsiella pneumoniae* (33.3%), *Citrobacter freundii* (33.3%). Study done by Bhandari p et al. confirmed ESBL in (32.25%) isolates; among them (25%) were *E. coli* followed by (20%) isolates of *K. oxytoca* and MBL production was found in 11 isolates; among them, 7 (63.8%) were *Acinetobacter* spp. followed by 2 (18.1%) isolates each of *K. oxytoca* and *K. pneumonia* [33]. Study by Parajuli NP et al. revealed (43.7%) of ESBL producer and *Escherichia coli* was major ESBL producer (70.9%) followed by *Citrobacter* species (62.5%) and *Klebsiella* (59.4%) The same study found (50.2%) MBL producer in which major MBL enzyme producers were *Acinetobacter* species (78.8%) [34]. Study done in Dhaka city showed that percentage of ESBL producer was (31.42%) [35]. Heavy administration of antibiotics in hospitalized patients and persistence of highly resistant strains could account for such a high finding of Multidrug resistance [36].

Conclusion

In our study (65.1%) samples Showed culture positivity. *Staphylococcus aureus* was the common causative agent of SSIs. Bacteria showed more than 50% resistance towards commonly used antibiotic like Cefexime, Ceftriaxone, Ciprofloxacin which is matter of concern. MDR rate was very high (89.5%) and we encountered (56%) MRSA, (23.7%) MBL and (21.3%) ESBL producer organisms. It is necessary for every medical practitioner to have better knowledge about causative agent of SSIs and their sensitivity pattern to control the wound infection and to minimize the rate of mortality and morbidity due to SSIs. A proper control of antibiotic usage will prevent the emergence of resistant strains of bacteria. Proper sterilization of surgical wards and surgical equipment we can reduce the rate of SSIs.

Recommendation

As Rate of isolation of organism causing SSIs is high, Samples should be collected from every suspected cases to find out the causative agent. Detection rate of MDR is high so AST should be done regularly for the selection of appropriate antibiotic in the treatment. To minimize SSIs proper sterilization of equipments and wards should be done time to time.

List Of Abbreviations

SSI: Surgical site infection

WHO: World Health Organizations

AMR: Antimicrobial resistance

MRSA: Methicillin resistant *Staphylococcus aureus*

AMR: Antimicrobial resistance

AST: Antibiotics sensitivity test

ESBL: Extended spectrum beta lactamase

MBL: Metall β-lactamase

TSI: Triple sugar iron agar test

SIM: Sulphide indol motility test

CLSI: Clinical and laboratory standard institute

CONS: Coagulase negative *staphylococcus aureus*

MDR: Multi-drug resistant

Declarations

Ethical approval and consent to participate:

The ethical approval for study was taken from Institutional Review Committee, Universal College of Medical Sciences (UCMS-TH) before sample collection.

Consent to publish:

Not applicable.

Availability of data and materials:

The raw data will be available on request.

Competing interests:

The authors declare they have no competing interests.

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Authors' contributions:

SK was responsible for study design, supervision of work, reporting and guidance. SK , SA , RP were contributed data analysis. SK, SA, RP, SR, RK, SLK , AP were contributed to writing and manuscript preparation. All the authors read and approved the final manuscript.

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Figures

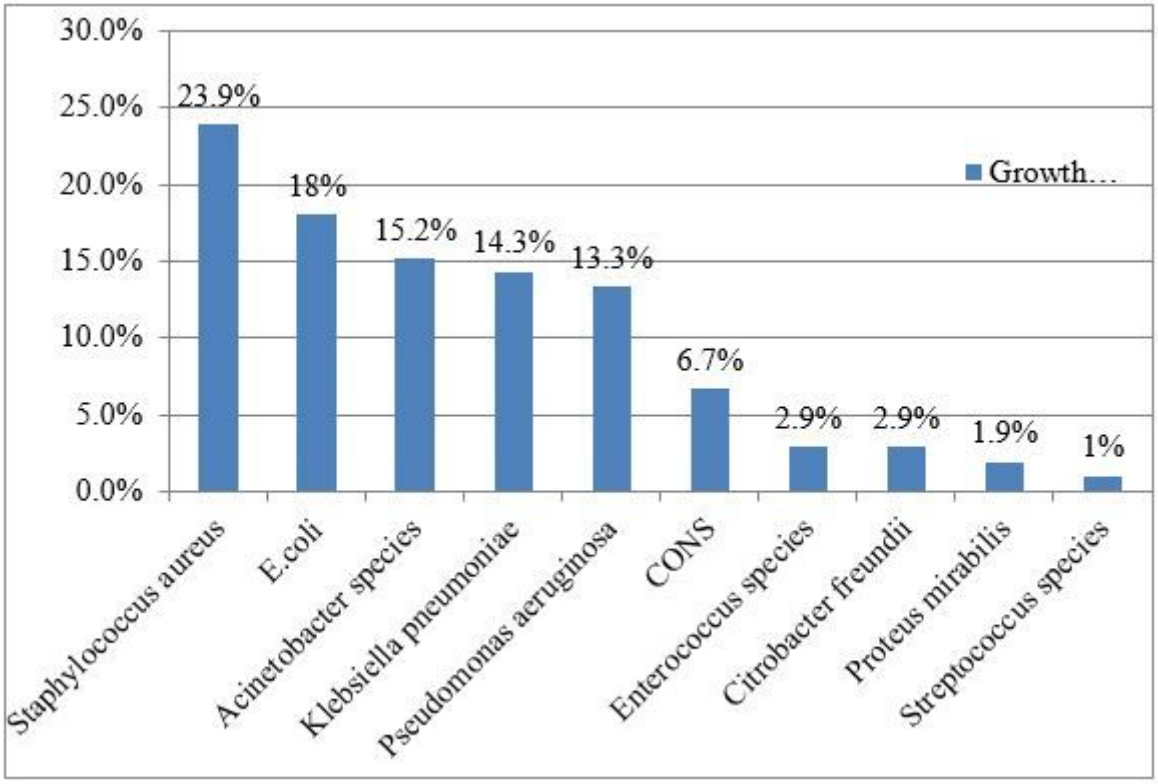

Figure 1

Pattern of bacterial isolates

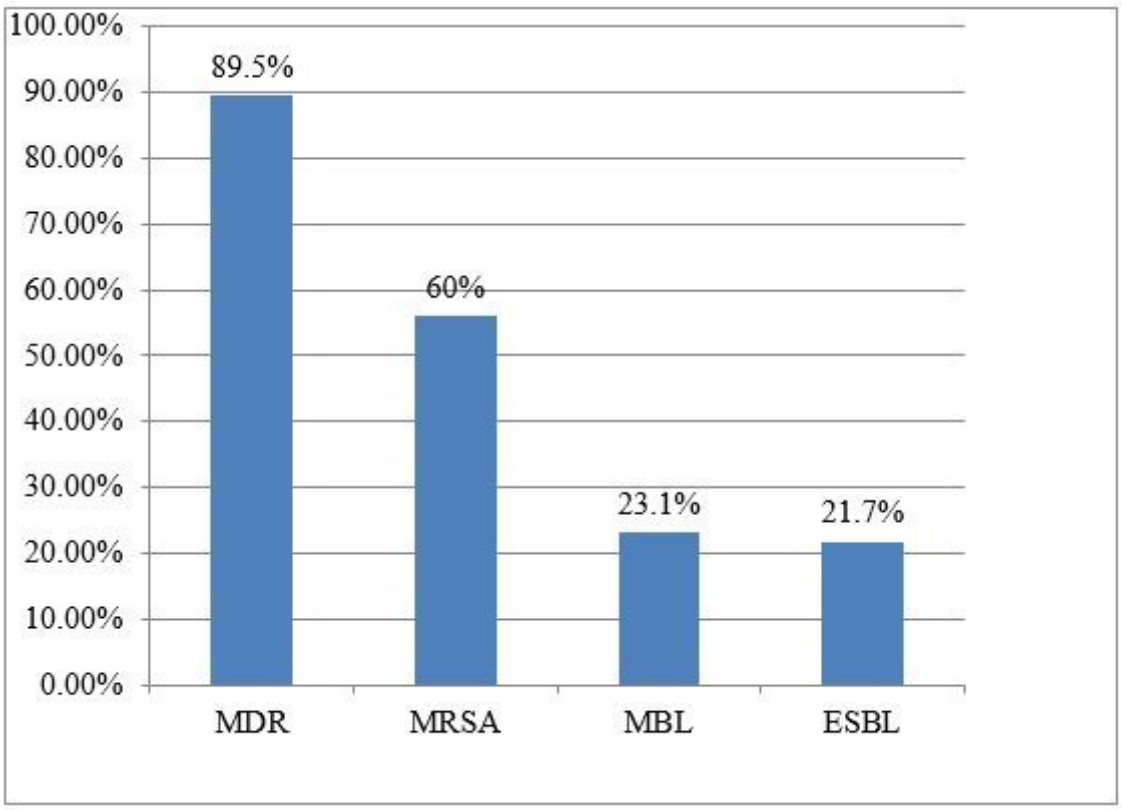

Figure 2

Pattern of MDR, MRSA, ESBL and MBL in isolated organisms