The Pharma Innovation Journal 2018; 7(7): 169-175

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(7): 169-175

www.thepharmajournal.com
Received: 17-05-2018
Accepted: 23-06-2018

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Post-operative wound infection: isolation, characterization and *in vitro* antibiotic susceptibility analysis

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Abstract
There is a fast emergence and vivid spread of multi-drug resistant super bugs in the hospital environment. Now a days, the drastic increase in the post-surgical infections are found due to various intrinsic and extrinsic factors. This investigation provide some idea about the prevalence of aerobic bacterial pathogens responsible for the post-operative wound infections and non-healing of the surgery induced wounds. A total of 50 post-operative cases with various surgeries and days of stay were recruited therby 65 bacterial isolates were characterized. Out of them, *Staphylococcus aureus* dominated followed by *Pseudomonas aeruginosa* and *Escherichia coli*. In the polybacterial determination, the combination of *Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* observed with maximum numbers. By determining the *in vitro* antibiogram, *Staphylococcus aureus* showed maximum resistant towards penicillin, tetracycline and tigecycline whereas, *Escherichia coli* showed resistance towards all antibiotics. One each of the isolates of *Pseudomonas aeruginosa* and *Proteus vulgaris* showed resistance to all antibiotics. Among the two isolates of *Klebsiella pneumoniae,* cefoxitin, etrapenem and ceftriazone are not effective and showed resistance. From this study, it was concluded that the appropriate usage of the antibiotics reduce the evolution of superbugs thereby nosocomial infections and drug failure cases will be reduced.

Keywords: Post-surgical wound infections, bacterial pathogens, antibiotic resistance

1. Introduction
Post Operative Infection (POI) is also known as Surgical Site Infection may substantially presenting for nearly 30 years after surgery, there is no other prosthetic are not available for this kind of infection [1]. In the field of surgery, post operative wound infection is the major issue, leads to various complicated problems which increase the morbidity and mortality rate [2-4]. Post operative infectionis the first line of infection creates after surgery and are mainly due to the patients' own oral pathogens, the secondary infection is initiated from the hospitals, but the type of the causative microorganisms may vary from hospital tohospital [5].The POI is highly depends on patients'defence system, condition and type of wounds and also the drugs prescribed to the patients [6]. The determination of etiological agent observed in the global epidemiology related to POI are observed as highly complicative mainly due to the lack of standardized diagnostic tools to measure the rate of infection. The lifethreatened evolution in POI is the emergence of antibiotic resistant microorganisms (super bugs), that may or maynot be controlled based on the biochemical and genetical interference leads to cure the infections by selecting the appropriate antibiotics (III and IV generation antibiotics). By maintaining the standard institutional antibiotic policy with interpretations of national and global system, mere reduction of such misuse, overuse, abuse of antibiotics and also drastic decrease in the emergence of superbugs may done [7]. To overcome such drastic situation, the healthcare workers including physician should be trained and updating for specific infections, practice of the proper infection control and notifying such situations. Due to lack of awareness, non-participation in orientation programmes, lesser documentation and improper implementation of policies providing false picture of post surgical epidemiology in developing countries [8].
The researchers of World Health Organization (WHO)documented high degree of POI in the developing countries than developed countries and are considered "Silent Killer" among nosocomial related diseases [9, 10]. Recently, a study stated that, *Staphylococcus aureus* (50.4%) was considered as predominant organism that cause POI, followed by *Escherichia coli*

(23.02%), *Pseudomonas aeruginosa* (7.9%) and *Citrobacter* species (7.9%) [11]. Isolates from gram positive groups showed maximum antimicrobial activity against several antibiotics includingvancomycin, teicoplanin, linezolid etc, whereas among gram negative groups showed sensitivity to meropenem, piperacillin, tazobactam, amikacin etc. The infectious agents isolated from the sites of post operative wounds are differ depending upon the underlying problems, location and type of surgical procedure etc [12, 13]. Although, the multidrug resistant pathogens are evolved in POI, the unbarable problems are Methicillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) [14, 15]. A common epitome for POI is that caused by *Pseudomonas aeruginosa* leads to varied insecondary infections due to its high antibiotic resistance. According to the National Nosocomial Infections Surveillance (NNIS) system, POI is the third most frequently recorded nosocomial infection and are documented about 12-16% among the hospitalized patients [17, 18].

Repeated mutations of the microbial pathogens due to the chemical exposure, physical mechanism like efflux pump and the selection pressure of antibiotics are inducing the microbes to drug resistant one [19]. In biochemical manner, the resistance is created by few mechanisms including inactivation of antibiotics, modification in the targets, membrane permeability and bypassing of the target [20-22]. The large evolution of bacterial resistance happens in the case of β-lactamase class and other group of antibiotics. Recently, more than 1000 resistant pathogenic strains have an ability to inactivate the antibiotics were recorded [22]. In genetics, the resistance is created by mutation in the different chromosomal loci or the obtaining of mobile genetic carrying resistance genes [23], which leads to irrespective response against the antibiotics and horizontal gene transfer method [22]. Due to the direct transmitting mechanism, the transfer of superbugs from human to human are happened by various routes like water and food.

The release and over exploitation of drugs including antibiotics in environment also play a major role in the exacerbation of antibiotic pollution and transfer the resistance genes to human commensal and other pathogens. In general, the waste water management system in hospitals and animal feed products having a wide number of antibiotic resistant microbes due to direct and indirect release of antibiotic residues in the environment. The misuse and unwanted usage of antibiotics in the clinical practice play a major phenomena in explorating newer and vibrant antimicrobial resistant strains of mono to multi drug varients [24, 25]. Thus the present study was planned to isolate the bacterial pathogens and also to determine the antibiotic resistant and sensitive nature of the isolates.

Materials and Methods

Study area and Participants

This study was conducted in the tertiary care teaching hospital located in the rural setup of Tiruchirapalli district of India. A battery of 50 patients admitted in the hospital for various illnesses and undergone for mild to severe surgery were included in this study. Sociodemographic, type of illnesses, type of surgery, hospital stay, antiseptics and antibiotics used for treatment were collected in a detailed proforma. The study was ethically certified by the institutional ethics committee and written informed consent was obtained from the patients during the time of interview. In case of unconsious cases, the consent was obtained from the attenders.

Sample Collection

A total number of 65 pus swabs were obtained from wound sites and the sample was collected using sterile cotton swab aseptically before the wound was cleaned (dressing done by using any antiseptic agents). In the ward itself, the swab was kept aseptically into the screw cap tube containing Nutrient Broth and transport to the Microbiology lab for processing. The samples were not allowed to stored for more than 6 hours. Automatically, the samples were rejected and discarded after the expiry time fixed by the laboratory.

Culturing of Sample

The collected samples were inoculated into Nutrient agar, Macconkey agar, Blood agar and Potato Detxrose agar (PDA) medium. All the inoculated plates were incubated at 37ºC for 24-48 hours; in the case of PDA, the plates were incubated at room temperature for 2-3 days. A control plates were also kept for comparative analysis and also to determine the sterility checking of the procedures done.

Confirmation of Isolates

All the bacterial and fungal isolates were identified by direct microscopy, colony morphology, gram reaction, motility test, biochemical tests including special confirmation tests. Further, the isolates were purified and subcultured periodically in respective agar slants and stored at low temperature until use. Whenever required, the cultures were dispensed in the respective broth cultures for confirmatory and antimicrobial susceptibility analysis.

In vitro antimicrobial susceptibility test

The isolates were further tested for analyzing antimicrobial susceptibility by standard Kirby-Bauer method. A battery of antibiotics with narrow and broad spectrum groups were impregnated for the analysis. A test bacterial cultures in broth were inoculated on the surface of Mueller Hinton agar plates. With known distance, the respective antibiotics were placed appropriately. The plates were incubated at 37ºC for 24 hours for the determination of antimicrobial susceptibility. The results were recorded as sensitive, intermediate and resistant according to the measurement of zone of inhibition by comparing with standard chart.

Results and Discussion

In this investigation, a total of 50 patients to undergone surgery and admitted in the post opeartive wards were included. The type of surgery was not included in this study. Among the subjects, maximum were found in between the age group of 41 and 70. The male population (86%) is more compared to female (14%) subjects. The age and sex wise distribution of the subjects were debicted in table 1.

Table 1: Age and Sex ratio among the subjects

Age limit	Male	Female
21– 30	8 (18.6)	-
31 – 40	6 (14.0)	3 (42.8)
41 – 50	11 (25.6)	1 (14.3)
51 – 60	6 (14.0)	2 (28.6)
61 – 70	10 (23.2)	1 (14.3)
71- 80	2 (4.6)	-
Total	43 (86)	7 (14)

[Figure in parenthesis denoted percentages]

The occupation based accidents and emergencies were noticed and recorded. Based on the medical record, maximum subjects underwent surgeries were mainly due to occupational based surgical interventions. Among the 50 subjects, 37 and 13 were from rural and urban background respectively. The occupational analysis among the patients were also done thereby, daily wages (43.2%) were maximum followed by farmers (21.6%) and drivers (13.6%). The detailed interpretation of occupation and residential status of the subjects were impregnated in table 2.

Table 2: Occupational and Residential details

Occupation	Rural (n=37)	Urban (n=13)
Farmers	8 (21.6)	-
Drivers	5 (13.6)	7(53.8)
Daily wages	16 (43.2)	2 (15.4)
Professionals	2 (5.4)	1 (7.7)
Students	3 (8.1)	1 (7.7)
Business	3 (8.1)	2 (15.4)

[Figure in parenthesis denoted percentage]

The data related to the subjects with animal contact were analysed thereby 31 out of 50 subjects had animal contact (Figure 1). The interrelationship between post surgical interventions and animal contact are not much matched. The literacy details of the subjects showed higher in secondary schoollevel (n=15) followed by illiterate group (n=13) and the data was described in figure 2. The annual income status of the subjects were anlaysed and depicted in table 3.

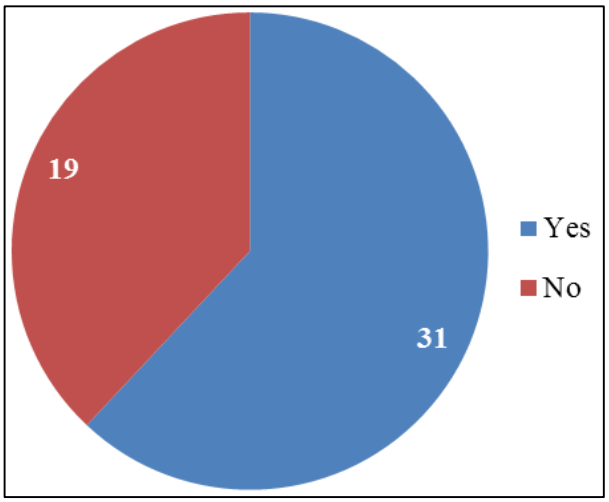

Fig 1: Details of animal contact

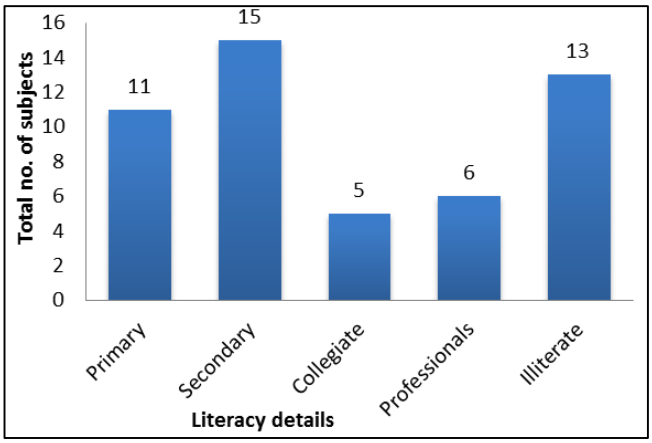

Fig 2: Comparitivenes of literacy among subjects

Table 3: Annual income details of subjects

Income in Rs	No of subjects	Percentage
10,000-25,000	15	30
25,000-50,000	4	8
51,000-1,00,000	8	16
1,00,000-1,50,000	11	22
1,50,000-2,00,000	7	14
Above 2,00,000	5	10

The history of diabetes among the subjects were recorded based on the medical record, patients' past medical data and current biochemistry. The investigation revealed 31 (62%) out of 50 patients are diabetic (Figure 4). The history of diabetics and the period of existence of blood sugar level were also interviewed with the patients and the data were impregnated in figure 5.

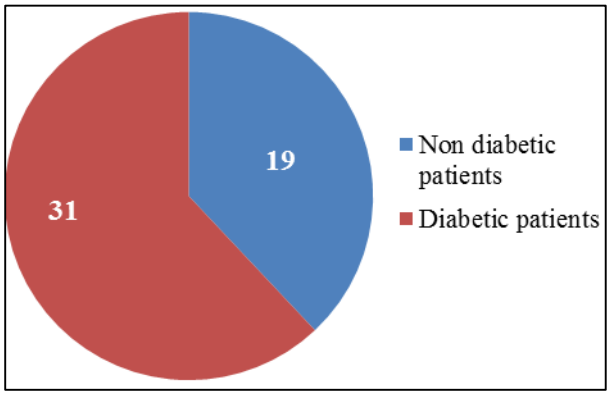

Fig 4: Diabetic Vs Non diabetic

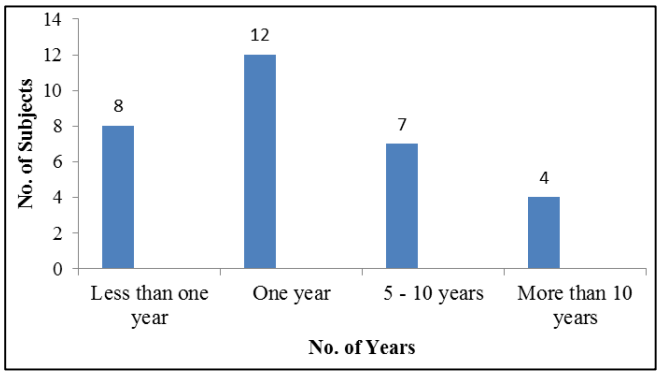

Fig 5: History of diabetes

As a continuation of the diabetic data analysis among the subjects, type of illness are also described and evaluated. As a result diabetic foot ulcer dominated with 24% followed by diabetic with cellulitis and ulcer with 22% and diabetic foot gangrene 16%. The other illnesses were mere and the details were tabulated (Table 4).

Table 4: Type of illnesses among subjects

Type of illness	Number of subjects	Percentage
Diabetic foot ulcer	12	24
Diabetic foot gangrene	8	16
Peripheral vascular disease	3	6
Abscess	5	10
Necrotizing Fascitis	4	8
Ulcer with varicose vein	7	14
Diabetic with cellulitis and ulcer	11	22

The nosocomial and iatragenic infection with various microbial sources play vital role in various infections including post operatice surgical complications related to infection. The number of days staying in the hospital also responsible for infection due to environment and induced mechanism. For that purpose, we analysed the number of days of hospital stay by the patients who underwent surgery and the day of sample collection (Table 5).

Table 5: Hospital stay in days and sample collection

No. of days	No. of patients	Day of sample collection
1-3	11 (22)	All the samples were collected before antibiotic administration
4-10	14 (28)	
11-15	7 (14)	
16-20	6 (12)	
20-25	4 (8)	
26-30	5 (10)	Samples collected at 7, 9, 12, 13, 15th day of the admission: one sample collected after antibiotic administration because of non healing wound
31-35	3 (6)	

As per the patients' examination and review chart, they are subjected to broad spectrum antibiotic(s) as a therapeutic initiative. Among the 50 subjects, 22 received mono antibiotic therapy (44%), whereas 28 received broad spectrum poly antibiotic therapy (56%). The detailed decription of a mono and poly antibiotic therapy with number of subjects received are impregnated in figure 6 and 7 respectively.

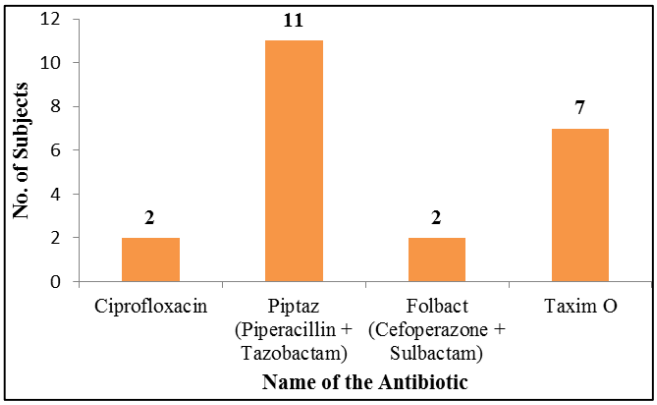

Fig 6: Mono antibiotic therapy

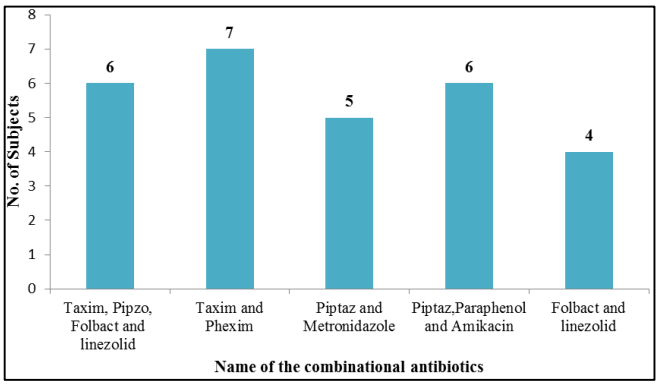

Fig 7: Poly antibiotic therapy

From the patients, wound and pus sample were collected aseptically in the bedside and the same were transferred for microbiological investigations. Thirty four (34) wound and 31 pus samples were collected from 50 patients in different locations and days (Figure 8). Out of 65, 14 samples had blood stain (Figure 9).

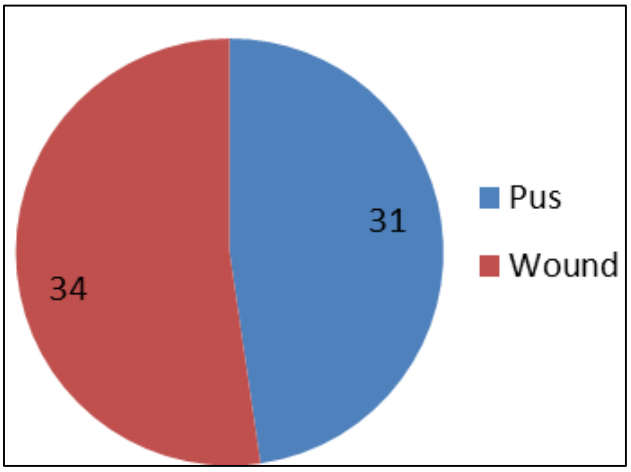

Fig 8: Type of samples

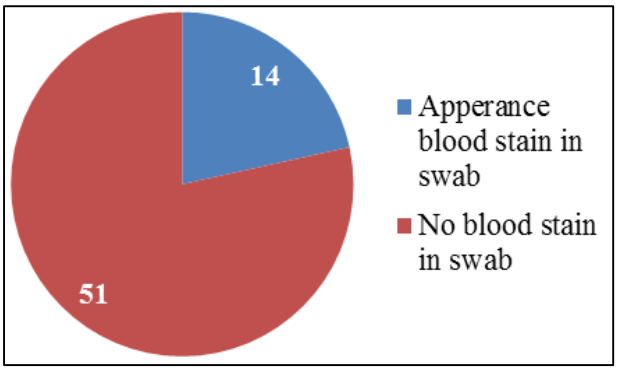

Fig 9: Appearance of blood stain

The bacterial pathogens isolated from the samples after appropriate microbiological procedure and characterized. Among the 65 samples, 61 supported bacterial growth and four showed negative (Figure 10).The overall bacterial isolates with the frequency of order were analysed thereby *Escherichai coli* dominated with 15 isolates followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* with 13 and 12 isolates respectively (Figure 11). In continuation of that, the assessment ofmonomicrobial and polymicrobial bacterial pathogens were characterized and recorded (Figure 12 and Table 6).

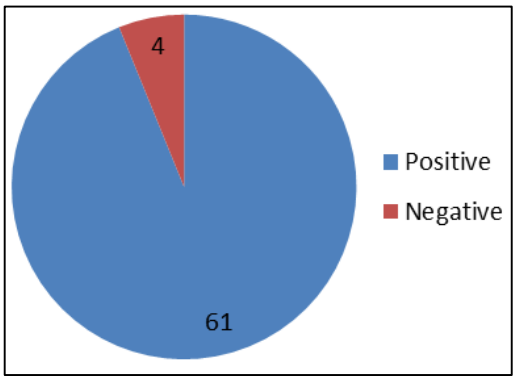

Fig 10: Bacterial culture positivity

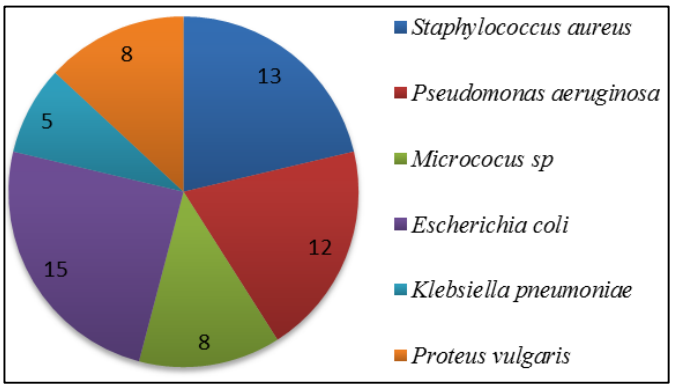

Fig 11: Overall bacterial culture

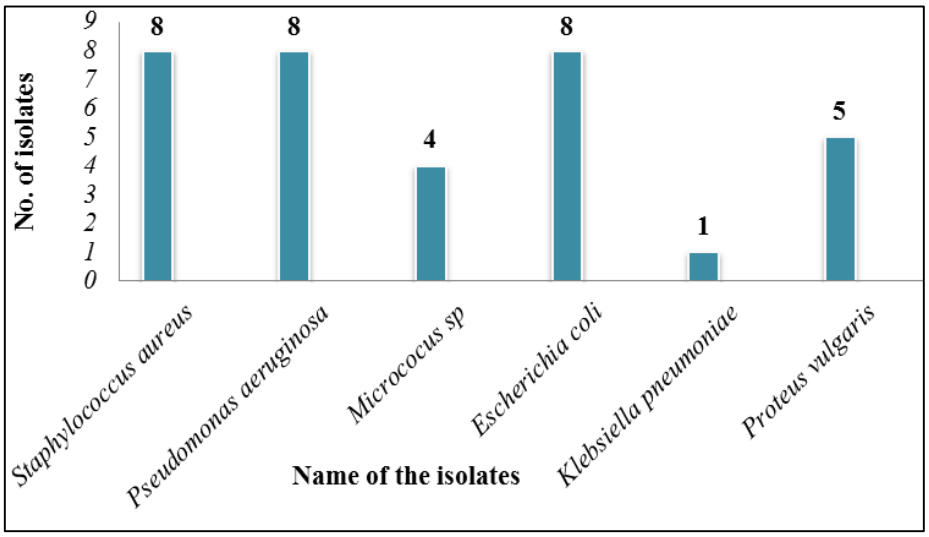

Fig 12: Monomicrobial bacterial isolates

Table 6: Polymicrobial bacterial isolates

Poly bacterial combinations	No. of poly bacterial isolates	Percentage
Staphylococcus aureus, Escherichia coli and *Pseudomonas aeruginosa*	3	11.1
Staphylococcus aureus, Escherichia coli and *Proteus vulgaris*	20	74
Escherichia coli, Klebsiella pneumoniae and *Micrococcus sp*	2	7.5
Pseudomonas aeruginosa and *Klebsiella pneumoniae*	1	3.7
Klebsiella pneumoniae, Proteus vulgaris and *Micrococcus sp*	1	3.7

The characterized bacterial isolates were further subjected to determine the antibiotic susceptibility by standard Kirby-Bauer method. The zone of inhibition of the isolates provided the information regarding sensitivity, resistance and intermediate response of bacterial isolates towards selected antibiotics. As a result, *Staphylococcus aureus* showed maximum resistant towards penicillin, tetracycline and tigecycline whereas, *Escherichia coli* showed resistance towards all antibiotics.

Among *Pseudomonas aeruginosa* and *Proteus vulgaris*, one of the isolate showed maximum resistance to all the antibiotics. Among the two isolates of *Klebsiella pneumoniae*, cefoxitin, etrapenem and ceftriazone are not effective and the isolates showed resistance. The detailed description of antibiotic susceptibility pattern of the bacterial pathogen against their respective antibiotics are depicted in table 7.

Table 7: Antibiotic susceptibility pattern of the bacterial pathogen

Antibiotic	Isolated organism (n=61) Vs Resistant pattern					
	***Staphylococcus aureus* (n=13)**	***Klebsiella pneumonia* (n=5)**	***Escherichia coli* (n=15)**	***Pseudomonas aeruginosa* (n=12)**	***Proteus vulgaris* (n=8)**	***Micrococcus sp.* (n=8)**
Amikacin	4 (30.8)	1 (20)	1 (6.7)	4 (33.3)	-	2 (25)
Amoxicillin	-	-	-	-	1 (12.5)	-
Ampicillin	2 (15.4)	-	1 (6.7)	-	-	-
Azithromycin	-	1 (20)	-	-	-	-
Cefixime	-	-	1 (6.7)	-	-	-
Cefoperazone	-	-	-	4 (33.3)	-	2 (25)
Cefotaxime	-	-	-	-	1 (12.5)	-
Cefoxitin	1 (7.7)	-	-	-	-	-
Ceftriazone	-	1 (20)	-	-	-	-

Cefuroxime	-	2 (40)	-	-	-	-
Ciprofloxacin	2 (15.4)	-	1 (6.7)	4 (33.3)	1 (12.5)	-
Erythromycin	2 (15.4)	-	-	-	-	1 (12.5)
Gentamicin	3 (23)	-	2 (13.3)	2 (16.7)	1 (12.5)	2 (25)
Imipenem	-	2 (40)	-	3 (25)	3 (37.5)	-
Nitrofuratoin	-	-	1 (6.7)	-	1 (12.5)	-
Ofloxacin	-	-	-	-	2 (25)	1 (12.5)
Penicillin	3 (23)	-	-	-	-	-
Sterptomycin	-	-	-	-	-	2 (25)
Tetracycline	1 (7.7)	-	-	-	-	-
Trimethoprin	-	1 (20)	-	-	-	-

[Figure in parantehsis denotes percentage]

The rate of wound infection in post-surgical environment are increasing nowadays and that may be due to variations in hospital acquired pathogens, non standardized policy of infection control and prophylaxis between hospitals. In this study, it was recorded that mono-microbial bacterial infections including *Staphylococcus aureus* (26%), followed by *Pseudomonas aeruginosa* (26%) and *Escherichia coli* (26%). Recent study in Kenya and Ethiopia also supported *Staphylococcus aureus* predominated, but instead of *Pseudomonas aeruginosa* in our study, *Klebsiella pneumoniae* be the second top predominant isolate [26, 27]. While analyzing polybacterial infections the combination of *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* observed maximum, whereas, the combination of *Proteus vulgaris* without *Escherichia coli* was observed in other study [28]. In this study, the isolation of aerobic bacterial pathogens was concentrated thereby, anaerobic pathogens excluded. On comparing with anaerobic surgical wound infection the confirmation of the importance of aerobes in surgical wound infection is accorded [29, 30].

According to the literature review, it was found that similar organisms have been reported from the isolation of various wound types. The prevalence of *Staphylococcus aureus* in surgical wound infections has been attributed to the high rate of nasal carriage in patients and healthcare workers who are involved in the treatment of patients [31]. *Pseudomonas aeruginosa* is considered as an epitome of opportunistic nosocomial bacterial pathogens, that are highly responsible for wide range of infections leads to chronic morbidity and mortality. The same was incidented in this study as *Pseudomonas aeruginosa* defined as second top nosocomial bacterial pathogen. Post-operative wound infections are at high risk after surgery and these originate due to poor post-operative management of surgical wound, longer hospital stay and continuous broad spectrum of antibiotic administration. These antibiotic therapy may be the major reason for the development of multi-drug resistant microbes.

This emergency healthcare alarm is an important call for a need in order to control poor hygenic practice, usage of low quality antimicrobial agents, prescription and consumption of unwanted and unprescribed antibiotics respectively. In practice, the usage of antibiotics are based on this chemical groups, mode of action and mechanism of resistance; thus choosing the antibiotics may have the policy based on appropriateness and availability in the hospital. In order to avoid the incidence of post-operative wound infection, the administration of peri-operative prophylaxis would have provide useful and effective empirical treatment. Based on the isolation (in single and multiple) and antibiotic resistance pattern, we also suggested and recommended to avoid indiscriminate use of antibiotics for reducing the increasing trends of antibiotic resistance globally. The regular surveillance should be carried out by the infection control committee of the health care institution for making the environment healthy and super-bug free. The formulation of standard appropriate antibiotic regimens for the treatment of surgical wound infections should be made by the institutions; severe action may take for the misusers of better patient management. A healthy discussion should be encouraged between microbiologist and the surgeons is strongly advised by this study, so as to prevent and control post-operative wound infection at minimal cost.

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