Resistance pattern of clinical isolates involved in surgical site infections

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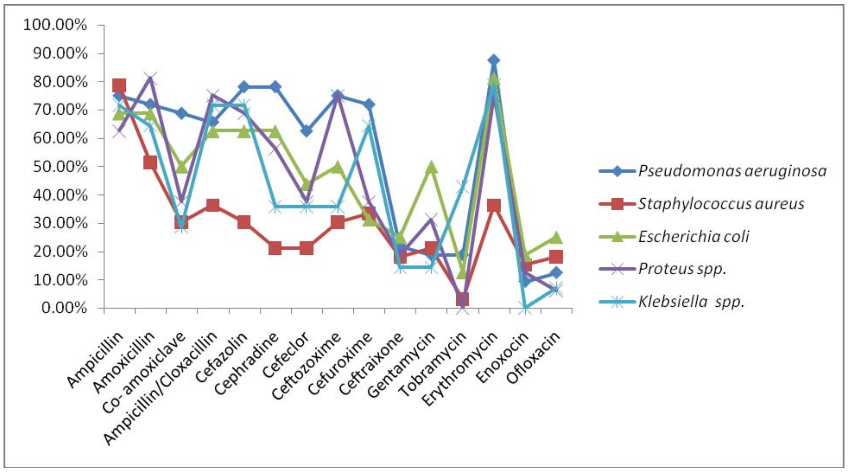
Graphical abstract:



Abstract: Wound infections due to the incursion of microbes need to be averted or to heal the wounds by antibiotics. Antibiotics are not only aid in cure of infections but also help to prevent the flourishing and production of one or more species of microorganism, resultant in purulent discharge.

This current study was carried out to evaluate the resistance pattern of clinical isolates from surgical site infections by the Kirby Bauer disc diffusion method. A total of 257 clinical isolates were collected from different hospitals in Karachi and evaluated by using fifteen antibiotics belonging to different groups. *Staphylococcus aureus* (n=87), *Escherichia coli* (n=76), *Pseudomonas aeruginosa* (n=56), *Proteus* (n=21) and *Klebsiella* (n=17) species are the most common clinical isolates of surgical site infections.

Among the semi-synthetic penicillins, ampicillin was found to be resistant to nearly all clinical isolates but amoxicillin was moderately sensitive to *S. aureus*. Combinations of semi-synthetic penicillins are more sensitive than the penicillin alone. Co-amoxiclave exhibits superior sensitivity to all the surgical infection isolates except *Pseudomonas aeruginosa* which showed 68.75% resistance. *Pseudomonas aeruginosa* was highly resistant to cephalosporin except ceftraixone which showed 21.88% resistance. *S. aureus* was slightly responsive to cefazolin, cephradine, cefaclor, ceftizoxime, cefuroxime and ceftriaxone. *E. coli*, Gram-negative clinical isolate was showed 25% and 31.25% resistance to ceftriaxone and cefuroxime. In the *Klebsiella* species, 71.42% and 64.29% resistance to cefazolin and cefuroxime respectively, was observed. Aminoglycosides such as gentamycin and tobramycin were found to be more susceptible to all the clinical isolates. Quinolones like ofloxacin and enoxacin were showed good sensitivity to nearly all the clinical isolates.

On the basis of the present study, it is recommended to adopt a rational use of antibiotics in prophylaxis and the utilization of a coordinated scheme of surgical wound inspections.

Keywords: Surgical site infections, clinical isolates, disc diffusion method, antimicrobial activity.

INTRODUCTION

Human beings are greatly concerned regarding the healing of wound infections**.** By the use of oily material, Egyptians were the first domain to heal up the wounds; Hippocrates used vinegar for the wetting of open wounds. The pioneer of infection control, Prof. Joseph Lister was the first who used carbolic acid (phenol) spray to control the microbial contamination in the operation room (Cohen, 1998). Wounds may be categorized as post-operative, pathological, and accidental (Collier, 2003). The Center of Disease control (CDC) of USA has amended the definition of wound infection to surgical site infection (SSI) to evade the confusion between infections of surgical contaminations and of traumatic wounds (Horan *et al*., 1992). In wound infections, there is flourishing incursion and multiplication of microbes resulting in purulent discharge.

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CDC has affirmed infections that may be occurred within 30 days of surgery or within a year in case of implants are considered as SSI (Weigelt *et al*., 1992). It has been established by many workers the cost of treatment increased due to the additional stay in hospital because of nosocomial infections (Cruse and Ford, 1980; DiPiro *et al*., 1998; Mangram *et al*., 1999; Leaper *et al*., 2004). A study has estimated the of cost around 2000 US dollar with approx 10 days stay additional (Cruse and Ford, 1980), while in Europe the stay in hospital is extended by 9.8 days at the cost of 325 euro (€) per day (DiPiro *et al*., 1998). SSIs has been considered as the second most frequent cause of nosocomial infections (Brachman *et al*., 1980), but due to the use of aseptic techniques and greater awareness, duration and timing of antibiotic administration, SSIs has now become the third most common nosocomial infection (Mangram *et al*., 1999). SSIs are always to be a great concerned for the health associated professionals, in 1964 National Research Council Group (NRCG) of USA has been first classified SSI based on microbial contamination (Berard and Gandon, 1964). Later on surgical wounds are defined as class I (clean), class II (clean-contaminated), class III (contaminated), and class IV (dirty-infected) (Dunn, 1994). But according to NNIS (1996) program, SSIs are classified as superficial incisional surgical site infection, deep incisional surgical site infection and organ/space surgical site infection (Mangram *et al*., 1999).

Antibiotics are commonly used in surgical prophylaxis for last three decades (Nichols, 2004). Pre-operative antibiotic prophylaxis has been reduced the occurrence of post-operative wound infections (Nichols, 2004; Mangram *et al*., 1999). Due to more bioavailability and rapid onset of action, the parental antibiotics are currently used in surgical prophylaxis (Nichols, 2004). Single dose of broad spectrum antibiotics are usually preferred in prophylactic measurements (Fonseca *et al*., 2006). The wide-ranging and irrational use of broad spectrum antibiotics has been doubled only with the span of six years with the increase of antimicrobial resistance (Craig, 1998), adverse reactions, and cost (Stanley *et al*., 1996). The use of pre-operative antibiotics within 2 hour before incision plays an important role in the reduction of SSIs (Stanley *et al*., 1996). With the span of time, microbes modify their traits to the antibiotics and convey their resistance qualities to their descendents (Craig, 1998). The resistance traits are developed due to suboptimal, irrational and extensive use (Knox *et al*., 2003), low dose antibiotics (Craig, 1998), long course of antibiotics (Sae-Tia and Chongsomchai, 2006), too early and too late administration (Sadique *et al*., 2009).

Regardless the administration of antibiotics in surgical prophylactic measurements, there are several risk factors which may play an important role in the incidence of SSIs colonization, obesity, pre-operative stay, blood transfusion, undernourishment, geriatric patients, duration of the operation, hair removing, smoking, oxygen, surgical attire, temperature, ultraclean air (Mangram *et al*., 1999).

It has been observed that the etiology of SSIs may differ from operation site (Giacometti *et al*., 2000). The distribution of pathogens involved in SSIs is similar in many countries. In a study of these infections in the European Union (EU), 27-40% were due to *Staphylococcus aureus*, 6-11% to *coagulase-negative Staphylococi*, 3-15% to *Escherichia coli* and 7-10% to *Pseudomonas aeruginosa* (Leaper *et al*., 2004). A study in Turkey showed that *S. aureus* accounted for 50% of 621 pathogens isolated from surgical site infections, *E. coli* for 8%, *S. pyogenes* and *P. aeruginosa* each for 7% and *coagulase-negative Staphylococci* for 6% (Surucuoglu *et al*., 2005). In Thailand, the most common causative pathogens identified in SSIs is *E. coli* (15.3%), *S. aureus* (8.5%), *P. aeruginosa* (6.8%), *Klebsiella pneumoniae* (6.8%) and *Acinetobacter baumannii* (3.4%) (Kasatpiba *et al*., 2005). In SSIs not only single genera of microbes has been isolated but also multiple genera of microbes has also been found.

Nearly all the investigators found *S. aureus* as the most frequent clinical isolate of SSIs (Lowy, 1998; Mordi and Momoh, 2009; Bode *et al*., 2010). Multiple organ failure may be leaded by cytokine which has been mainly produced by many Gram-negative bacteria (Mangram *et al*., 1999; Demling *et al*., 1993). *P. aeruginosa* are mainly found with *S. aureus* especially in cardiovascular surgery. Due to highly resistance in *P. aeruginosa* as compared to other vegetative bacteria, it is mainly associated with highest death tools and has been found in a range of respiratory equipments. *Proteus* species are found ubiquitously (Tinne *et al*., 1967) and can be abridged by personnel and environmental sanitation (Mordi and Momoh, 2009). *Klebsiella* species has upside edge over other bacteria and one of the most important clinical isolate in nosocomial infection of SSIs (Jarvis and Martone, 1992).

MATERIALS AND METHODS

Collection of specimens

Two hundred and fifty seven clinical isolates belonging to different genera were isolated from post-surgical pus, purulent discharge or wound exudates. *S. aureus, P. aeruginosa, E. coli and Proteus* and *Klebsiella* species were collected on sterile swabs. Clinical isolates were obtained from patients who had developed sign and symptoms of SSIs, from different hospitals and pathological laboratories of Karachi. The isolates were identified based on their colony characteristics on different media and confirmed by biochemical reactions. The isolates were inoculated in caso agar/tryptic soya agar slants. These slants had been preserved at 4^oC in the refrigerator. Anti microbial resistance (AMR) has been determined by Clinical and Laboratory Standard Institute (CLSI, formally NCCLS) standards and by Kirby-Bauer disk diffusion method (Bauer *et al*., 1966; CLSI, 2006).

Preparation of inoculums

Muller-Hilton broth (MHB) was used to prepare inoculums and matched with McFarland standard. All tubes were incubated at 37^{o}C for few hours to develop the required turbidity as that of the McFarland standard. Muller- Hilton agar (MHA) was used to determine the sensitivity of clinical isolates. Bauer, Kirby, Sherris and Tuck were strongly suggested Mueller Hinton agar for performing antibiotic susceptibility tests by using single disk of high concentration (Bauer *et al*., 1966).

Inoculation of bacterial culture

A sterile swab was dipped into a broth suspension of bacterial culture. Excess inoculum was removed by rotating the swab against the inside wall of the tube with slim pressure. The whole surface of MHA plate was then streaked uniformly in three directions approximately at 60^{o} angle from each other. The lid was then replaced and the plates were allowed to dry for 10-15 min.

Placement of antibiotic disc

The appropriate antibiotic impregnated discs were placed on the agar surface with sterile forceps. Each disc was pressed down gently with the forcep to assure good contact with agar surface. The disc should be distributed such that each is at least 24 mm from center to center of its closer neighbor and 12 mm from the edge of plate.

Incubation

The plates were overturned within 15 min of placing the disc on agar and incubated at 35-37^{o}C for 24 hours. After incubation the diameter of the clear zones around the antibiotic disc were measured by using vernier caliper. All the bench work was carried out near a flame to create a zone of inhibition of invading bacteria and maintained the integrity of medium.

RESULT

Surgical site infections are of great concerned to the surgeons and to the health-care professionals. SSI is one of the major causes of infections difficult to control and handle. It has been observed by many workers that the cost of treatment is increased due to over stay in the hospitals due to super and nosocomial infections (Sheng *et al*., 2007; Mahmoud *et al.*, 2009; de Lissovoy *et al.*, 2009; Broex *et al.*, 2009). The resistance pattern of clinical isolates involved in SSIs may vary from one hospital to another hospital, surgeon to surgeon, procedure to procedure and from patient to patients (Nichols, 2001). In the present study, fifteen different antibiotics of diverse classes were evaluated against the pathogens which are the most commonly and frequently occur in SSIs (NNIS, 1996; Ali *et al*., 2009;**)**, for example *S. aureus, E. coli, P. aurerginosa, Proteus* and *Klebsiella* species (Table 1).

In the present study, ampicillin and amoxicillin were found 78.79% and 51.53% resistance to *S. aureus* but tobramycin, enoxacin showed 3.03%, 15.15%, respectively while ceftriaxone and ofloxacin exhibited 18.18%.

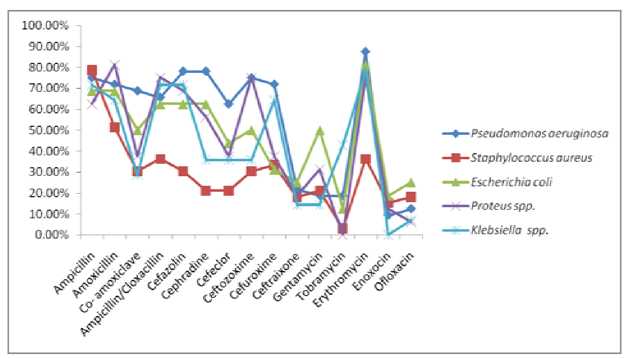
The sensitivity of *E. coli* with tobramycin 12.5%, Enoxacin 18.75% and ofloxacin and ceftraixone were 25%. The present study was found *E. coli* was resistant to cefuroxime, gentamycin and amoxicillin 31.25%, 50% and 68.75% correspondingly.

In the current study, *P. aureginosa* was found 87.50% resistance to erythromycin, 78.13% to cefazolin and cephradine, 75% resistant to ampicillin and ceftraixone.

Proteus species were highly resistant to amoxicillin 81.25% while about 75% resistant to ceftixozime, erythromycin, ampiclox, cefcalor and 68.75% to cefazolin. It was highly responsive to tobramycin 100%, ofloxacin 93.75% and enoxacin 87.5%.

The resistant was found to erythromycin 78.57% while ampicillin, cefazolin and ampiclox were showed 71.42% resistance, on the other hand highly susceptible to enoxacin 100%, oflxacin 92.86% but to gentamycin and ceftriaxone found 85.71%**.**

The resistances of microbes isolate from SSIs against antibiotics are shown in table 1 and fig. 1.



DISCUSSION

According to several investigators (Lowy, 1998; Mordi and Momoh, 2009; Bode *et al*., 2010) *S. aureus* is the most often isolated pathogen involved in SSIs. Cephalosporin is broadly used in prophylaxis by surgeons due to well tolerability and excellent result oriented against the isolates involved in SSIs (Munckhof, 2005). It has been reported that cefuroxime has exhibited superior activity against *S. aureus* (Onche and Adedeji, 2004). Due to the wide use of cephalosporins**,** *S. aureus* is now

Table 1: Resistance Pattern of clinical isolates involved in SSIs

Antibiotics	Microorganism				
	Pseudomonas aeruginosa	*Staphylococcus aureus*	*Escherichia coli*	*Proteus* spp.	*Klebsiella* spp.
Ampicillin	75.00%	78.79%	68.75%	62.50%	71.42%
Amoxicillin	71.86%	51.53%	68.75%	81.25%	64.29%
Co-amoxiclave	68.75%	30.30%	50.00%	37.50%	28.57%
Ampicillin/Cloxacillin	65.63%	36.36%	62.50%	75.00%	71.42%
Cefazolin	78.13%	30.30%	62.50%	68.75%	71.42%
Cephradine	78.13%	21.21%	62.50%	56.25%	35.71%
Cefeclor	62.50%	21.21%	43.75%	37.50%	35.71%
Ceftozoxime	75.00%	30.30%	50.00%	75.00%	35.71%
Cefuroxime	71.88%	33.33%	31.25%	37.50%	64.29%
Ceftraixone	21.88%	18.18%	25.00%	18.75%	14.29%
Gentamycin	18.75%	21.21%	50.00%	31.25%	14.29%
Tobramycin	18.75%	3.03%	12.50%	0%	42.86%
Erythromycin	87.50%	36.36%	81.25%	75.00%	78.57%
Enoxocin	9.38%	15.15%	18.75%	12.50%	0%
Ofloxacin	12.50%	18.18%	25.00%	6.25%	7.14%

increasing the resistance against cephalosporins, like cefuroxime was 33.33% and ceftizoxime and cefazolin were 30.30% resistant.

The current study was supported by Mordi and Momoh (2009) in which *E. coli* was found as the closest contender to *S.aureus*. Giacometti (2000) found *E. coli* was defiant to ampicillin 66%, cefazolin 90.6%, ceftriaxone 90.6% and co-amixiclave 96.2%. But in the present study, *E.coli* was 68.75%, 62.5%, 50%, and 25% resistant to ampicillin, cefazolin, co-amoxiclave and ceftriaxone accordingly.

In nosocomial infections, *S. aureus* was notable found with *P. aureginosa* (Giacometti *et al.*, 2000). *P. aeruginosa* was the one of most frequently isolated nosocomial pathogen (Pollack, 2000; Adwan *et al.*, 2009; Lister *et al.*, 2009). *P. aeruginosa* has transmitted its high resistance to their new generation and associated with high death tools. A study conducted by Giacometti *et al*. (2000) showed that *P. aureginosa* was 56% susceptible to co-amoxiclave in 1995, 52.9% in 1997 and 57.75 in 1999 while cefazolin was 100% sensitive in these years but in case of ceftraixone 56.1%, 58.8% and 64% susceptible pattern in year 1995, 1997 and 1999 respectively. *P. aeruginosa* has the ability to survive on minimal nutrition requirement and physical tolerability. A variety of respiratory support apparatus is unhygienic due to moisture content and offers an ultimate growth of organism particularly *P. aeruginosa. P. aeruginosa* can survive from six hours to 16 months on dry, inanimate surfaces in hospitals. It is also mainly found in respiratory therapy equipment, antiseptics, soap, sinks, mops, medicines, and physiotherapy and hydrotherapy pools (Lister *et al.*, 2009). *P. aeruginosa* was showed good sensitivity against enoxacin and ofloxacin, 9.38% and 12.5% respectively and with tobramycin and gentamycin which possesses anti-pseudomonal activity around 18.75% the results were differentiated from the statement of Poole (2005).

Proteus species were found in all over environment in food, intravenous solutions, contaminated water, on the skin of human-being. Its prevalence in Ghana was 9.8%-14.6%. *Proteus* species were resistant to in general by used antibiotics leading to a higher prevalence of resistant bacteria (Feglo *et al.*, 2010). *Proteus* species were defensive to erythromycin (Mordi and Momoh, 2009) but responsive to gentamycin (Ahmed *et al*., 2009).

Klebsiella species were opportunistic bacteria found on mammalian mucosal linings, and on the surface of body of human and in the environment. *Klebsiella* species were one of the most frequent opportunistic pathogens mainly involved in nosocomial infection of SSIs (Acheampong *et al.*, 2011). *Klebsiella* species were 71.42% resistant to amoxicillin supported by Livrelli *et al*. (1996).

It has been suggested that there must be an alliance of the surgeons, pharmacist, epidemiologist and microbiologist to adopt their restricted infecting susceptible pattern into an account to intend prophylactic and as well as empirical treatment guideline for individual surgical site (Ali *et al*., 2009). The greatest problem which is common in all types of surveillance is the patients, who develop infections at home (Smyth and Emmerson, 2000). The surgeons must rigorously fellow the guidelines of empiric therapy to evade and to tend and treat SSIs. It cannot be possible that guidelines of developed country like U.K (NICE) and USA (FDA) provided full and through information of the resistance pattern of isolates involved in SSIs for every region of world. It is the prime responsibility and strongly recommended by WHO that the local bodies has to be prepared their empiric guideline to start antibiotic therapy and it is better and supported by various studies to perform culture sensitivity of the SSI patients.

Due to unrelenting defiance, WHO, CDC, NICE and surgical infection society were solidly suggested the day to day surveillance plan in SSIs. SSI is one of the most troubled issue and firing alarm for health concerned professionals. SSIs are the burning reason of health associated infection and main cause of morbidity and infections like respiratory and urinary tract infections, bacterimias and antibiotic associated diarrheas and even it may lead to death.

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