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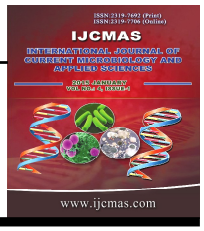

Original Research Article

Antibiotic resistance profiles of bacteria isolated from surgical wounds in tertiary hospitals, Tanzania

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Keywords

Surgical sites, Infection, Imprint Technique, Antibiotic resistance

A B S T R A C T

This study intends to isolate the most common bacteria-associated with surgical site wound infections using imprint method owing to its large wound surface coverage, embedment of surgical site fluid and being cost-effectiveness, and assess their sensitivity patterns to commonly used antibiotics using the Kirby-Bauer disc diffusion method. A total of 62 patients who underwent surgical procedures from three tertiary hospitals were recruited, from whom a total of 158 bacteria were isolated; that is multiple infections were common among the patients. *Staphylococcus aureus*, *Pseudomonas, aeruginosa*, *Proteus mirabilis* and *Enterobacter aerogenes* were the most frequently encountered bacteria. About 55% of the isolated bacteria were resistant to the antibiotics. Of all tested antibiotics, ciprofloxacin and co-trimoxazole were the most and least effective against both Gram negative and Gram positive bacteria. The imprint technique has proved to be feasible and useful for surgical site wound microbial sampling for resources-limited health settings. Hence we recommend routine surgical site wound sampling prior antibiotic prescription to prevent further spread of antimicrobial resistance.

Introduction

A surgical wound infection can develop at any time from two to three days after surgery until the wound has healed (usually 2 to 3 weeks post operation). Occasionally, an infection can occur several months after an operation (Humphreys, 2009; Anderson, 2011). Most surgical wound infections are limited to the skin, but can spread to deeper tissues as consequence of microbial invasion/infections, of which majority are caused by human normal flora. Most chronic infections of wounds are colonized with different microorganisms, especially problematic bacteria like antimicrobial-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus*, which represent an increasing therapeutic challenge in wound management. It is therefore essential to specify the bacteria in surgical wounds for an individual-specific treatment. Wound due to physical trauma, burn or surgical procedure predisposes the

internal body tissues to microbial infections, especially bacterial ones. Wound infection impairs the healing process. In case of severe wound infection, healing becomes more difficult and sometimes converts into chronic wounds. For surgical wounds, if such a situation occurs, re-suturing may become necessary (Cheadle, 2006).

Surgical site infections (SSI) and infections of other types of wounds due to physical trauma or burns are difficult to prevent particularly in health facilities (Wong, 2004; Naqvi et al., 2013). The SSI are common sources of morbidity among operated patients, which are normally diagnosed on clinical criteria rather than microbiological criteria, as wounds may be colonized by microbial species without any adverse effects on healing process (Cheadle, 2006). Therefore, routine microbiological investigation is not justified (Hansonn et al., 1995). Nevertheless, laboratory investigation provides clinicians with important information of types of microorganisms present in a wound and their antibiotic susceptibility, which is helpful for successful wound management (Brook et al., 2013). A major challenge during treatment of SSI is resistance of the pathogens to empirically prescribed antimicrobial agents. The empirical mode of treatment leads to the pathogens to develop antimicrobial resistance, which may result into non-healing of the SSI and making the patient even more ill and occasionally causes death.

Despite the presence of diverse wound sampling techniques, the consequences of SSI such as morbidity, long hospitalization stay and/or polypharmacy for SSI management are still increasing (Fehr et al., 2006; Akoko et al., 2013). In most patients, an exemplary bacterial swab is taken from the center of the wound surface (Ghazal et al., 2012). However inadequate skills and costs incurred to perform the current wound swab sampling techniques may hinder their routine application for treatment of SSI. Therefore, present study highlights the use of imprint technique for surgical wound sampling and subsequent sensitivity testing of clinical bacteria associated with wound infections.

Materials and Methods

Patients and Sample collection procedures: Patients who were undergone surgical procedures at Muhimbili Orthopedic Institute (MOI), Mwananyamala Municipal Hospital (MMH), and Amana Municipal Hospital (AMH) were recruited for this purpose. The Whatman filter paper No.1 (size of 3 x 4 cm) was employed as microbial transmission medium. The pre-sterilized Whatman No. 1 filter paper was aseptically attached to 24 hours blood agar plate. The filter paper was brought into contact with a surgical wound surface for 10 seconds then removed back on to the fresh blood agar and transported to microbiology laboratory. Then the filter paper was incubated at 37 ºC for 48 hours in 10 % CO_2 (in a candle jar).

Isolation and identification of the isolates:

Following 24hours of incubation of wound derived filter paper on the blood agar; the resultant bacteria were sub-cultured on McConkey agar (MCA) in order to purify the observed microbial isolates. The prepared MCA plates were then incubated at 37^{o}C for 24 hours and then subjected to identification process using the conventional methods (Cheesbrough, 2006). The obtained pure bacterial colonies were sub-cultured separately on the Nutrient agar (slants) prior to subjecting them to antimicrobial susceptibility testing.

Antibiotic susceptibility testing procedures: The identified bacterial isolates were tested against 8 commonly used antibiotics: ampicillin (30µg), Erythromycin (15µg), Ciprofloxacin (5µg), Tetracycline (30µg), Ampiclox (30µg), Gentamycin (10µg), Cotrimoxazole (25µg) and Chloramphenicol (30µg). The Kirby–Bauer disk diffusion method was employed and antibiotic susceptibility results were interpreted as per Clinical Laboratory and Standards Institute (CLSI, 2006). Two strains of reference bacteria from the American Type Culture Collection (ATCC) namely *Escherichia coli (*ATCC25922) and *Staphylococcus aureus* (ATCC25923) that are conserved in the Laboratory of Microbiology/Immunology-School of Medicine (MUHAS) were employed as control bacteria. Each microorganism was sensitized by sub-culturing into freshly prepared Mueller-Hinton broth (Roth, Germany); then turbidity of each bacterial suspension was compared to that of McFarland 0.5 standard turbidity (equivalent to 1.5×10^8cfu/ml); prior to performing antibiotic susceptibility profiling as per CLSI guidelines (CLSI, 2006). Following an overnight incubation at 37°C, diameters of inhibition zones (IZ) were determined in millimeter.

Ethical consideration: The study was granted ethical clearance by the Muhimbili University of Health and Allied Sciences Ethical Committee and responsible authorities at MOI, MMH and AMH. Prior commencement of samples collection, both verbal and written informed consents were sought from each participant, who was also vividly explained the study's objectives. Patients were also informed that all the information gathered and laboratory findings would be used for research purpose, but could be available for any participant who would wish to know the findings. In order to maintain confidentiality data were entered into the computer for analysis and interpretation by using code numbers. Specific ethical issues like pain during imprint sampling were addressed with assurance that pain was self limited and in case of persistence, analgesics would be offered.

Results and Discussion

A total of 62 patients who underwent surgical procedures at MOI, AMH and MMH were recruited in this study. Slightly less than half (47.55%) of the patients were from MOI, simply because is the National Orthopedic Institute that offers such specialized services (Table 1). From the 62 study participants' surgical sites/wounds, a total of 158 pathogenic bacteria were isolated, which means occasionally, from a single surgical site more than two bacteria were also isolated. Most of the SSI-associated bacteria were comprised of *Staphylococcus aureus* (STA) and *Proteus mirabilis* (PRO) as shown in Table 2.

Comparison of the sensitivity profiles of the isolated bacteria with respect to reference strains of bacteria namely *S. aureus* (Ref-STA) and *E. coli* (Ref-ECO) that represent the Gram positive and negative bacteria respectively, showed statistically significant differences between the pathogens and the strains of reference bacteria ($p<0.05$) as indicated in Figures 1 & 2. Antibacterial susceptibility tests of the isolated pathogens to commonly prescribe and used antibiotics revealed that only 45(27%) of the pathogens were susceptible to the antibiotics, while 87(55%) were resistant. More than half of the tested isolates of *Staphylococcus aureus, Streptococcus pyogenes, Entereobacter aerogenes, Proteus mirabilis* and *Pseudomonas aeruginosa* exhibited resistance to the antibiotics (Table 3).

Ciprofloxacin (CIP) was the most effective antibiotic against Gram negative bacteria with exception to KLE species. High sensitivity was observed among KLE, SAL, and BAC isolates against C30 (Figure 2). On the other hand, SXT25 was not effective against ENT, PRO and ECO (Figures 1 and 2). However, placid effects of SXT were observed against SAL as shown in (Figure 2). Majority (60%) of STR isolates was resistant to E15, TE30 and ACL30, similarly over 50% of STA isolates were resistant to E15 (Figure 1 and Table 3). SXT was the least effective antibiotic against KLE, SAL, PSE and BAC. Moreover, high variability of sensitivity to SXT25 was observed in SAL isolates (Figure 2).

Surgical site infection is the second most common health facility acquired infections after urinary tract infection and contributes significantly to morbidity and mortality in patients. Several previous studies conducted in Tanzania indicate that the rate of SSI has been increasing over the past 3 decades (Fehr et al., 2006; Akoko et al., 2013). Presumably, because aseptic techniques employed in developing countries for preparing patients for surgical procedure or for cleaning and dressing surgical site wounds are not effective enough to assure aseptic conditions.

Techniques for obtaining specimens from surgical site wounds include wound swabbing, needle aspiration and wound tissue biopsy, which could be either costly or require special skills. The imprint technique employed in our study produced reliable samples for microbiological analysis and proved to be more cost-effective. Our results revealed presence of *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli* and *Enterobacter aerogens*, which are not very different from those previously isolated by Signoretti et al., (2013) and Reiffel et al., (2013).

The present study show that the antimicrobial resistance rate of various bacteria infecting human is on increase. Majority of the isolated bacteria (especially Gram negative rods) showed high resistance rate to SXT25. The antibiotic is one of the most frequently employed for prophylaxis of opportunistic infections such as Pneumocyst jiroveci pneumonia, toxoplasmosis and other parasitic infections in HIV/AIDS patients (Geresu et al., 2014; Zhu et al., 2014, Nowaseb et al., 2014). Resistance of *E. coli* isolates to SXT is also of major health concern as the antibiotic is widely used for prophylaxis and management of diarrheagenic *E. coli* infections in immunological compromised individuals (Omran et al., 2014). Our present study shows that empiric antibiotic treatment should be broad as the aetiology can be polymicrobial (mixed aerobic–anaerobic microbes) or Gram negative and Gram positive bacteria (Stevens et al., 2014). Four of nine types of the isolated bacteria were human normal flora, which implies that some hygienic measures or /and aseptic surgical procedures were breached. Moreover, the presence of a large number of bacteria in a single surgical site buttresses the above observations.

Most of the bacteria that exhibited higher rates of antibacterial resistance are human normal flora and biofilm forming pathogens such as *S. aureus, P. aeruginosa, E. aerogenes* and *Streptococcus spp*. *Pseudomonas aeruginosa* causes infection in all parts of the human body. The bacterium is naturally resistant to a wide range of antibiotics, which is attributable to its resistance mechanisms such as efflux pumps

and the ability to form biofilm that reduces further *P. aeruginosa's* susceptibility to antibiotics. The presence of such biofilm greatly contributes to persistent bacterial infections in surgical sites because of their inherent high tolerance to all antimicrobials and immune cells (Alhede et al., 2014). Clinicians must understand the role of pathogenic biofilms that play in impairing the healing of chronic SSI and in increasing the risk for wound infection, as our results show implication of most human flora in the surgical sites/wounds. The susceptibility of pathogenic biofilm in the SSI and their antimicrobial resistance depend on their composition, virulence, bioburden and host's patho-physiology and most importantly immunological status (Waldrop et al., 2014; Bugli et al., 2014; Christensen et al., 2014). Moreover, exposure of inner tissues by skin puncture (wounds) and traumas associated with wounds may greatly disturb the local inflammatory responses, increasing vulnerability to bacterial infections (Naqvi et al., 2013; Buikema et al., 2014; Shekarabi et al., 2014). Sampling of SSI and susceptibility testing prior prescription of antimicrobial agents may significantly minimize irrational use of the agents and reduce further spread of antimicrobial resistance; through such intervention, highly virulent bacteria with unpredictable antibiotic susceptibility profiles can be determined (Brook et al., 2013; Anderson, 2011).

Conclusively, high antimicrobial resistance was revealed among the SSI-associated bacteria. Ampicillin-derivatives (AM30 and ACL30) and SXT24 were the least effective against the Gram positive and Gram negative bacteria respectively. Ciprofloxacin remains the most effective antibacterial agent for both Gram positive and Gram negative bacteria. We recommend that sampling of SSI using the imprint technique and sensitivity testing prior antimicrobial prescription may greatly prevent further spread of antimicrobial resistance.

Table.1 Bacteria isolated from surgical wound sites

Bacteria	**Frequency of bacterial isolation/ Hospital**		
	MOI (%)	AMH (%)	MMH (%)
Staphylococcus aureus (STA)	12 (7.6)	2(1.25)	15(9.5)
Streptococcus pyogenes (STR)	3(1.9)	2(1.25)	5(3.2)
Enterobacter aerogenes (ENT)	11(7.0)	1(0.6)	14(8.9)
Proteus mirabilis (PRO)	14(8.9)	3(1.9)	10(6.3)
Escherichia coli (ECO)	6(3.8)	0(0.0)	8(5.0)
Klebsiella pneumoniae (KLE)	3(1.9)	2(1.25)	7(4.4)
Salmonella typhi (SAL)	3(1.9)	1(0.1)	2(1.25)
Pseudomonas aeruginosa (PSE)	12(7.6)	2(1.25)	11(7.0)
Bacillus sublitis (BAC)	4(2.5)	2(1.25)	3(1.9)
Total	68 (43.0)	15(9.5)	75 (47.5)

Table. 2 Number of bacteria isolated from the surgical wound in the tested patients

Total No. of bacteria isolated/ patient	Bacterial Species								
	STA	**STR**	**ENT**	**PRO**	**ECO**	**KLE**	**SAL**	**PSE**	**BAC**
1	1	0	0	5	0	1	0	0	0
2	2	2	1	2	0	1	0	2	1
3	16	4	11	11	9	4	2	3	0
4	6	1	5	5	3	2	3	15	3
5	3	2	4	1	1	0	0	3	2
7	1	1	3	0	1	3	0	1	0
11	0	0	2	3	0	1	1	1	3
Total	**29**	**10**	**26**	**27**	**14**	**12**	**6**	**25**	**9**

Table. 3 Antibiotic sensitivity patterns of bacteria from surgical wound sites

Bacteria	**Susceptibility Status**		
	Sensitive	Intermediate	Resistant
STA	6	7	16
STR	2	2	6
ENT	6	4	16
PRO	7	5	15
ECO	5	3	6
KLE	4	2	6
SAL	3	1	2
PSE	6	3	16
BAC	4	1	4
Total	45(27.2%)	28(17.7%)	87(55.1%)

Fig. 1 Sensitivity profiles of reference strains and the isolated bacteria

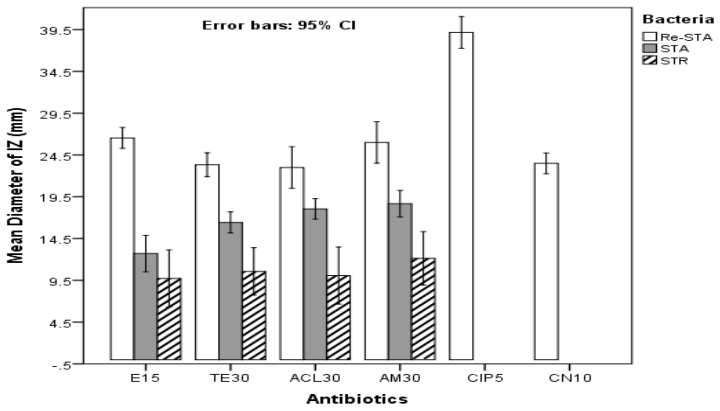
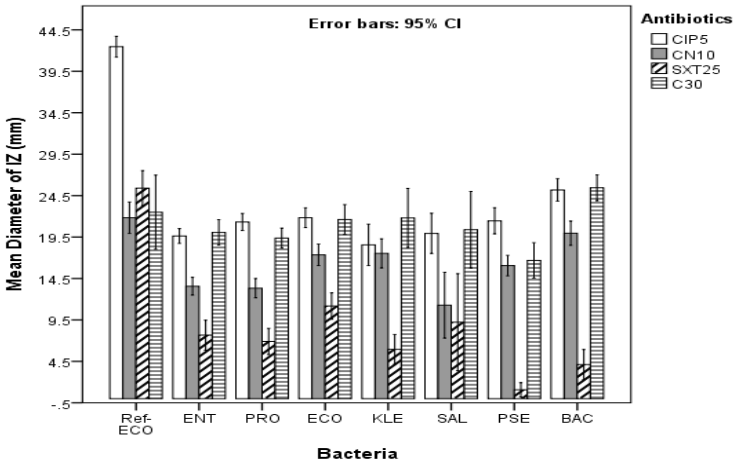


Fig. 2 Sensitivity profiles of Gram negative bacteria and reference strains of *E. coli*



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References

Akoko, L. O., Mwanga, A. H., Fredrick, F., and Mbembati, N. M. 2011. Risk Factors of Surgical Site Infection at Muhimbili National Hospital, Dar es Salaam, Tanzania. *East Central Afr J Surg*, 17(3):12-17.

Al Ghazal, P., Körber, A., Klode, J., Schmid, E. N., Buer, J., and Dissemond, J. 2014. Evaluation of the Essen Rotary as a new technique for bacterial swabs: results of a prospective controlled clinical investigation in 50 patients with chronic leg ulcers. *Int Wound J*, 11(1): 44-49.

Alhede, M., Alhede, M., and Bjarnsholt, T. 2014. Novel Targets for Treatment of Pseudomonas aeruginosa Biofilms. In *Antibiofilm Agents* (pp. 257-272).

Anderson, D. J. 2011. Surgical site infections. *Infect Dis Clinic North Am*, 25(1):135-153.

Brook, I., Wexler, H. M., and Goldstein, E. J. 2013. Antianaerobic antimicrobials: spectrum and susceptibility testing. *Clin Microbiol Rev*, 26(3), 526-546.

Cheadle, W.G. 2006.Risk factors for surgical site infection. Surg Infect 7(1):S7-S11.

Cheesbrough, M. 2006. District Laboratory Practice in Tropical Countries. Part 2, Second Edition. Cambridge University Press The Edinburgh Building, Cambridge, CB2 8RU, UK.

Christensen, G. J. M., and Brüggemann, H. 2014. Bacterial skin commensals and their role as host guardians. *Beneficial Microbes*, *5*(2): 201-215.

CLSI-Clinical Laboratories Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. Wayne, PA: CLSI; 2006.

Fehr, J., Hatz, C., Soka I., Kibatala P., Urassa, H., Battegay, M. 2006. Antimicrobial prophylaxis to prevent

surgical site infections in a rural sub-Saharan hospital. *Clin Microbiol Infect*;12:1224-7.

Geresu, B., Misganaw, D., and Beyene, Y. 2014. Retrospective evaluation of cotrimoxazole use as preventive therapy in people living with HIV/AIDS in Boru Meda Hospital. *BMC Pharmacol Toxicol*, 15(1), 4.

Hansson, C., Hoborn, J., Moller, A., Swanbeck, G. 1995. The microbial flora in venous leg ulcers without clinical signs of infection. *Acta Derm Venereol*, 75(1): 24-30.

Humphreys H. 2009. Preventing surgical site infection. Where now? J Hosp Infect, 73: 316-322.

Naqvi, S. H. A., Naqvi, S. H. S., and Coetzee, E. 2013. Pseudomonas aeruginosa burn wound infection in a dedicated paediatric burns unit: letters. *South Afri J Surg*, 51(4), 151-152.

Omran, E. A., Mahafzan, A. M., and Shehabi, A. A. 2014. Antimicrobial resistance patterns of diarrheagenic and non-diarrheagenic Escherichia coli isolates from Libyan children. *Inter Arabic J Antimicrob Agents*, 4(1).

Reiffel, A. J., Pharmer, L. A., Weinstein, A. L., and Spector, J. A. 2013. A prospective analysis of the association between indwelling surgical drains and surgical site infection in plastic surgery. *Ann Plastic Surg*, 71(5), 561-565.

Robson, M.C. 1997. Wound infection. A failure of wound healing caused by an imbalance of bacteria. *Surg Clin North Am*, 77(3): S1-S3.

Shekarabi, M., and Asgari, F. 2014. Aging Immunity and Infection. In *Immunology of Aging* Springer Berlin Heidelberg, pp. 231-238.

Signoretti, F. G., Gomes, B. P., Montagner, F., and Jacinto, R. C. 2013. Investigation of cultivable bacteria isolated from longstanding retreatment-resistant lesions of teeth with apical periodontitis. *J Endodontics*, 39(10), 1240-1244.

Stevens, D. L., Bisno, A. L., Chambers, H. F., Dellinger, E. P., Goldstein, E. J., Gorbach, S. L.,and Wade, J. C. 2014. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. *Clin Infect Dis* (2):e10-e52.

Waldrop, R., McLaren, A., Calara, F., and McLemore, R. 2014. Biofilm Growth Has a Threshold Response to Glucose in Vitro. *Clin Orthop Related Res*, 1-6.

Wong, E.S. 2004. Surgical site infection. In: Mayhall CG, editor.Hospital Epidemiology and Infection Control. Philadelphia:Lippincott Williams & Wilkins,pp 287-310.

Zhu, Q., Wang, L., Lin, W., Bulterys, M., Yang, W., Sun, D. and Wang, Z. 2014. Improved Survival with Co-Trimoxazole Prophylaxis among People Living with HIV/AIDS Who Initiated Antiretroviral Treatment in Henan Province, China. *Current HIV Res*, 12(5), 359-365.