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Original Paper

Detecting Nosocomial Intrinsic Infections through Relating Bacterial Pathogens of Incision

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ABSTRACT

Surgical procedures often lead to both intrinsic and extrinsic infections. In order to improve on recovery of patients, investigations were carried out on samples collected from patients during and after surgery. Laboratory analysis was performed on wound swabs from incision, colon segments, scrapes, tissues, pus and catheter specimen urine. The samples were cultured on MacConkey and Blood agar and incubated aerobically at 37^0C for 16-24 hours. Thereafter, isolates were identified using standard microbiological methods. Results showed that isolates from wound were also found on endogenous indicators of surgery. *Klebsiella* species from incision was 15 (18.75%) while those from colon segment was 30(37.6%), scrapes 8(16%) and pus 3(7.5%). *Acinetobacter* species found on incision was 15(7.5%) and pus 7(2.3%). *Pseudomonas* species was distributed on incision 5(2.5%), colon segment 4(5%), tissue 3(1.6%), scrapes 5(10%) and pus was 5(12.5%). *Staphylococcus aureus* which was isolated from incision was 2(1%), while scrapes and pus were 5(10%) and 7(17.5%) respectively. Catheter associated urinary tract infections yielded significant bacteriuria (64.7%), almost twice the rate of non-significant bacteriuria (35.3%); indicating the need to remove all catheters as soon as possible. Antibiogram of isolates of *Klebsiella pneumoniae* with resistance pattern: ApGnNaNt, *Escherichia coli* (ApCtNaTtCm) and *S. aureus (*ApChCxErPn) with plasmid sizes in the range (30.2-52.51Kb) were common to both indicators and wound, showing that the pathogens were the same clusters. This study demonstrated surgical procedures as precursory to intrinsic infections and that bacterial pathogens found on wounds and endogenous indicators of surgery are links to intrinsic infection. The study therefore emphasizes the need to culture wounds promptly to effect speedy recovery of patients who have undergone surgery.

Key Words: Bacterial pathogens, Endogenous indicators, Nosocomial infection, Surgery

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INTRODUCTION

Micro-organisms from intrinsic and extrinsic sources have been known to cause nosocomial infections (CDC, 1991). The human body enables survival of a wide variety of microorganisms with potential for causing infection (Burke, 1961). In circumstance where systemic host resistance is lowered, such as immunosuppression from medication, disruption of intact cutaneous or mucous membrane as a result of surgical procedures or trauma, patients' bacterial flora may become opportunistic and cause infection (Donowitz *et al*., 1982; Koontz, 2000).

Reports have shown that surgical site infection may be connected to intrinsic infection and this has become a major concern among surgeons and infection control practitioners.

The incidences of nosocomial infection vary by body site, which is determined to a large extent by underlying disease conditions in the patients who are exposed to high risk medical interventions including surgical operations and invasive devices. An important aspect in the initiation of nosocomial infections is the opportunistic microorganisms from the patient's body, which contribute to the flora of

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the hospital environment especially patients on frequent admission for surgery (Horan *et al*., 1993; Ho *et al*., 1995; Alsaimary and Mezaal, 2009).

The Centers for Disease Control (CDC) in 1988 modified the term 'wound' to specify surgical site and anatomic location of deep infection. The distinction between this component of surgical site and incisions is important in the pathogenesis of surgical site infection (SSI) following certain operative procedures (Medina *et al*., 1996; Biedenbach *et al.*, 2004). Wound connotes incision from skin to deep soft tissue while organ/space defines any part of anatomy (e.g. organ or spaces) other than the incision opened or manipulated during operative procedures (Aganovic *et al.*, 1994).

Attempts made to reduce infection at wound sites classified wounds into four categories, clean, clean-contaminated, contaminated and dirty wound (Cruse and Foord, 1973). From wound stratification, Wishnewski *et al*. (1998) reported *Staphylococcus aureus* as the common isolate from clean surgery while *Escherichia coli* dominated in cultures from infected wounds. Reports from CDC (1988) and Hani and Adnan (2009) recorded *Pseudomonas aeruginosa* as the most commonly isolated pathogens of surgical infection. From another study, *P. aeruginosa* and *S. aureus* isolated from critically ill patient suggested colonisation from endogenous rather than exogenous sources (Kropec *et al*., 1993; Shriyan *et al.*, 2010).

In this present study however, incision and indicators of surgery were investigated for bacterial pathogens in order to establish intrinsic source of wound infection often seen in surgical patients and to deduce methods to reduce them at the Lagos University Teaching Hospital, Idi Araba, Lagos, Nigeria. It is hoped that findings from the study will serve as base for infection control in tertiary hospitals.

SUBJECTS, MATERIALS AND METHODS

Subjects and Specimen Types

A total of two hundred (200) patients undergoing surgery of various kinds at the modular theatre of the Lagos University Teaching Hospital (LUTH) were examined during the study. The operative procedures included colon surgery, laparatomy, appendectomy, orchidectomy, prostatectomy, other genito-urinary procedures, orthopaedic, caesarean section, myomectomy, other obstetrical procedures and thoracic surgery. Three main types of specimens were collected: excised tissues (colon, urologic scrapes, gynaecologic fibroid and pus), wounds (were swabbed before closing incision and samples collected later on the ward after 3-4 days) and catheter specimen urine (CSU) (collected immediately after operation, a follow up was done thereafter; urine was also collected 3-4 days in the ward).

Laboratory Analysis of Specimens

Specimens were transported in stuarts transport medium while urine samples were collected in sterile sterilin bottles and transported to the research laboratory immediately. Specimens for aerobic culture were inoculated unto Blood Agar (BA) and MacConkey Agar (MA) (Oxoid CM 61) and thereafter incubated at 37^0C for 16-24 hours. Urine samples were processed by carrying out microscopy and culture. Standard laboratory methods were used to identify and characterise isolates (Cowan and Steel, 1993; Farmer, 1995). Urine cultures which yielded ≥20 (5×10^4) colonies forming unit (CFU) were classified as significant bacteriuria.

Qualitative Bacteriology

All aerobic isolates were Gram stained and all Gram negative organisms were tested for citrate, gas production, indole, lysine decarboxylase, urease, motility and hydrogen sulphate production. Further tests were then carried out on a series of carbohydrate utilisation which consisted of glucose, arabinose, lactose, mannitol, rhamnose, trehalose and xylose. The Gram-positive cocci were tested for catalase and coagulase production (Cowan and Steel, 1993).

Typing of Bacterial Isolates

Both phenotypic and genotypic methods were used to type the isolates:

Antibiogram

An antibiogram was carried out as an initial screening test using a disc diffusion method. Inoculum was prepared by adding 3-4 colonies of bacteria into sterile normal saline in screw-capped tubes. Turbidity was standardised by comparison with 0.5 MacFarland standard. Using sterile cotton wool swabs, Mueller Hinton Agar (MHA) plates were swabbed with the suspension (Woods and Washington, 1995, Bauer *et al.*, 1996). Gram-positive and Gram-negative discs (DT-POS and DT-NEG, Biotec LTD, England) were then applied. The Gram positive discs consisted of Ampicillin (25µg),

Tetracycline (20µg), Cloxacillin (10µg) and Gentamycin (10µg). The Gram-negative antibiotics discs were Ampicillin (30µg), Cotrimoxazole (25µg), Gentamycin (10µg), Nalidixic acid (20µg), Nitrofurantoin (30µg), Colistin (10µg), Streptomycin (25µg) and Tetracycline (50µg). The third generation cephalosporins included were Ceftrixone (30µg), Cefuroxime (30µg) and Ceftazidime (30µg) (Oxoid, Clinpath Limited, Basingstoke, Hampshire, England) (Bauer *et al.*, 1996)

Plasmid Analysis

All isolates with similar antibiotic profile were analysed for plasmids by a modification of the technique of Forbes *et al*. (1991). Isolates were grown overnight in selective broth and spun in microcentrifuge at 12,000rpm for 10 minutes. The supernatant was poured off, vortexed to loosen pellet, followed by the addition of solution A (1.32M potassium acetate, solution B (0.1M NaOH, 1% SDS) and solution C (10mM Tris HCl in 1mM EDTA). Electrophoresis was carried out on 0.8% agarose gels. Gels were stained with ethidium bromide (0.5µ/ml) for 45 minutes. Thereafter, photographs were taken. *Escherichia coli* V517 of molecular weight standard (2.2 – 55.7kb) served as control. The plasmid analysis was done at Molecular Biology and Biotechnology Division of the Nigerian Institute of Medical Research, Yaba, Lagos.

Statistical Analyses

Data analyses were done by using EPI Info 6.0. Statistical significance was drawn by X^2 – test. When values tended towards 0.001, they were shown to be significant.

RESULTS

Relationship between Microbial Load of Incision and Endogenous Indicators of Surgery

Various types of bacteria were distributed on endogenous samples from patients, who had undergone surgical procedures (Table 1). The highest isolated pathogens were *Klebsiella* species, 30(37.5%) and *E. coli* 20 (35%) from colon segments. 15 (7.5%) of *Acinetobacter* species found on the incision were linked to 7(2.3%) of those found on tissue. Other pathogens isolated were as follows: *Elkenella corrodens* 20(10%) found on incision were related to 5(1%) tissues. *Bacillus species* 7(3.5%) on incision were related to 6 (12%) on scrapes. *Pseudomonas aeruginosa* 5(2.5%), *S. aureus* 2(1%) and coagulase negative *Staphylococcus* 17(18.5%) which were isolated from incisions were related to those found on colon, segment, scrapes, tissues and pus by antibiogram.

Table 1: Microbial Load of Incision and Endogenous Indicators of Surgery

Microbial Growth	Incision 200 (%)	Colon Segment 80 (%)	Scrapes 50 (%)	Tissues 30 (%)	Pus 40 (%)
Klebsiella spp.	15(18.75)	30 (37.5)	8 (16)	0 (0)	3 (7.5)
Escherichia. coli	10(17.5)	20 (35)	2 (4)	5 (16.7)	2 (5)
Enterobacter spp.	5(6.25)	10 (12.5)	4 (8)	0 (0)	0 (0)
Providencia spp.	0 (0)	5 (6.3)	2 (4)	0 (0)	3 (7.5)
Morganella morganii	0 (0)	2 (2.5)	7 (14)	0 (0)	4 910)
Proteus mirabillis	0 (0)	7 (3.5)	6 (12)	3 (10)	5 (12.5)
Acinetobacter spp.	15 (7.5)	0 (0)	0 (0)	7 (2.3)	0 (0)
Eikenella corrodens	20 (10)	0 (0)	0 (0)	5 (1)	0 (0)
Moraxella spp.	0 (0)	0 (0)	0 (0)	2 (6.7)	3 (7.5)
Bacillus spp.	7 (3.5)	0 (0)	6 (12)	0 (0)	0 (0)
Pseudomonas aeruginosa	5 (2.5)	12 (15)	7 (14)	0 (0)	2 (5)
Pseudomonas spp.	5 (2.5)	4 (5)	5 (10)	3 (10)	5 (12.5)
Burkholderia cepacia	0 (0)	3 (3.8)	4 (8)	1 3.3)	2 (5)
Staphylococcus aureus	2 (1)	0 (0)	5 (10)	0 (0)	7 (17.5)
Coagulase negative *Staphylococcus* (CONS)	17 (8.5)	5 (6.3)	2 (4)	0 (0)	5 (12.5)
Anaerobes	0 (0)	7 (3.5)	2 (4)	0 (0)	2 (5)
No growth	134 (67)	0 (0)	0 (0)	18 (60)	0 (0)

Table 2: Nosocomial Bacterial Isolates Found in the Theatre and on the Ward

Pathogen	Isolates from theatre	Isolates from ward	Total (%)
Klebsiella pneumonia	17	47	64 (6.4)
Enterobacter sp.	10	18	28(2.8)
Escherichia coli	8	23	31(3.1)
Citrobacter freundi	4	23	27 (2.7)
Serrata marcescens	2	16	18 (1.8)
Pseudomonas aeruginosa	27	66	93 (9.3)
Proteus mirabilis	8	31	39 (3.9)
Providencia rettgeri	2	11	13 (1.3)
Morganella morganii	1	11	12 (1.2)
Acinetobacter sp.	11	37	48(4.8)
Alkagenes sp.	2	25	27 (2.7)
Staphylococcus aureus	20	64	84(8.4)
Coagulase negative *Staphylococcus*	12	35	47(4.7)
Enterococcus faecalis	1	5	6(0.6)
Yeast	0	5	5(0.5)
Eikenella corrodens	2	14	16(1.6)
Rosemonas Sp.	1	3	4(0.4)
Burkholheria cepacia	3	7	10(1.0)
Cedecea neterui	0	6	6(0.6)
Kingella kingae	0	2	2(0.2)

X^2 1215; Df 19; $p<0.05$; $x^2 = 2382.717$, df 95.9 <0.05

Table 3: Comparison of Isolates from Urinary Catheter in the Theatre and Patients in the Ward

Surgical Specialty	Organisms from theatre	Organisms from wards	Isolation rates in theatre (%)	Isolation rates in wards (%)	Duration of catheter (in days)
General surgery (50)	Pr	Pr, PS	15 (30)	35(70)	≥ 5
Cardio-thoracic (10)	Sm	Mm, Sm	2(20)	(8(80)	≥20
Orthopaedic (25)	—	Ps, Kb	0(0)	25(100)	≥ 10
Urology (50)	Mm,Y	Ec, Mm, Y, Sr	20(40)	30(60)	≥ 40
Paediatric surgery (30)	Ps	Ps, Kb, Sr	5(17)	25(83)	≥ 30
Obstetrics and Gynaecology (35)	Ec	Ec, Kb	10(9)	8(23)	≥ 4

Abbreviation: Pr – Proteus, Pv – Providencia, Ps – Pseudomonas, Ec- Escherichia coli, En – Enterobacter, Kb – Klebsiella, Sm – Serratia, Mm – Morganella morgani, Y-Yeast, Sr – Streptococcus, Ak – Alkaligenes

Relationship between Bacterial Pathogens found in the Theatre and on the Ward

The different organisms isolated on the ward were significantly higher than those isolated in the theatre (Table 2). *Pseudomonas aeruginosa* was the highest pathogen from patients on the ward and theatre with 66 and 27 isolates respectively followed by *Staphylococcus aureus* with 64 (9.3%) and 20 (8.4%) respectively. *Kingella kingae* was the least isolated pathogen from patients on the ward with 2(0.2%) isolates. Statistical analysis showed that rates of isolation from the theatre and ward were significant ($P<0.05\%$) and a *df* of 95% indicated higher rates of infection as immunosuppression increased.

Relationship between Catheter Specimen Urine and Urinary Pathogens

There was a direct link of isolates from catheter urine specimens from the theatre with those isolated on the ward. For general surgery patients with duration of catheter ≥5 days, *Proteus sp* and

Pseudomonas sp were isolated within the range of 30-70% each from theatre and ward. Isolates which included yeast, *Morganella morganii, E. coli,* and *Pseudomonas sp* were within 40%-60% from urology patients from theatre and ward, which was significant for endogenous source of pathogens (P<0.05). The 60% and 83% of catheter associated urinary infection from urology and paediatric patients were also significant, p<0.05 (Table 3). Some procedures had higher rates of bacteria implicating the catheter as an associated cause of urinary tract infection. Genitourinary manipulation had 77.3% (17/22) significant bacteriuria, whereas paediatric patient had 69.2% (18/26). Those who had appendectomy done were least with 16.7% (1/6), (p<0.05). In general, significant catheter - associated bacteriuria was 216 (64.7%) while non-significant bacteriuria was 118 (35.3%) (Table 4).

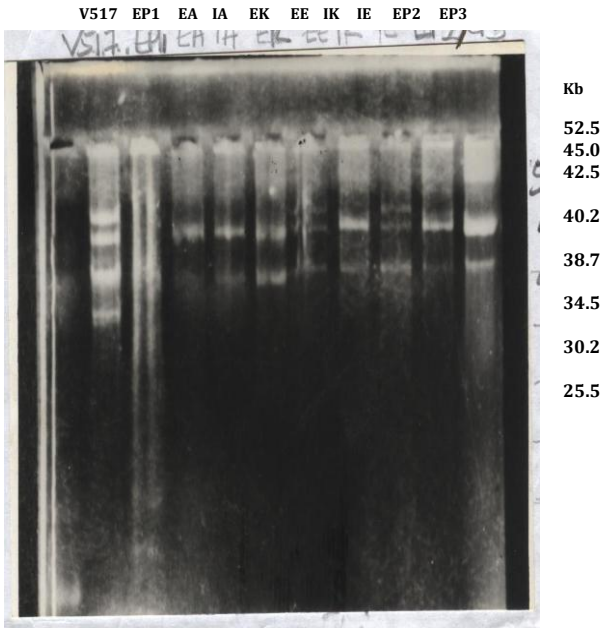

Figure 1: Plasmid Profile of Bacterial Pathogens Isolated from Indicators of Surgery and Incisions

V517 (Standard strain) EP1 (Endogenous Pseudomonas), EA (Endogenous Acinetobacter) similar to IA (Incision Acinetobacter), EK (Endogenous Klebsiella) similar to IK (Incision Klebsiella), EE (Endogenous E.coli), similar to IE (Incision E. coli), EP2 (Endogenous P. aeruginosa) similar to IP3 (Incision P. aeruginosa)

Relationship between Isolates by Antibiotypes and Plasmid Patterns

Resistance patterns of *Klebsiella pneumoniae*: ApGnNaNtCt, *Enterobacter sakazakii*: ApNaCtTt, *Escherichia coli*; ApGnNaSt and *Staphylococcus aureus*; ApChCxErPn were common to strains found on endogenous indicators as well as incisions. Some of the related bacterial pathogens had similar plasmid patterns (Figure 1; Table 5). Isolates with greater than 60% resistance to antibiotic tested expressed similar plasmid patterns. Four strains of *Klebsiella pneumoniae* from endogenous indicators had similar resistant profile (ApGnNaNtCt) to six strains from incision. *Enterobacter* sakazakii with resistant profile, ApCtGnNaNt, had three of the patterns from endogenous indicators similar to six patterns from incision. Two strains of *Pseudomonas aeruginosa* from endogenous indicator exhibited the same resistant profile (ApClCm) as those from incision (Table 5). While large plasmids were associated with *Klebsiella* spp and *Pseudomonas*, *Acinetobacter* spp displayed smaller bands. The plasmid sizes ranged from 25.7 – 52.5 Kb (Figure 1).

Table 4: Catheter-associated Urinary Tract Infection in Surgical Procedures

Procedure	Non - significant bacteriuria	Significant bacteriuria	Total
Appedenctomy	5 (83.3)	1 (16.7)	6
Caesarean section	29 (40.3)	43 (59.7)	72
Cholecystectomy	1(100)	0 (0)	1
Colon surgery	6 (33.3)	12 (66.7)	18
Gastric surgery	2 (66.7)	1 (33.3)	3
Genitourinary	5 (22.7)	17 (77.3)	22
Herniorrhapy	4 (100)	0 (0)	4
Laparatomy	11 (27.5)	29 (72.5)	972.5
Liver/spleen	0 (0)	1 (100)	1
Mastectomy	11 (44.0)	14 (56.0)	25
Myomectomy	4 (66.7)	2 (33.3)	6
Obstetrics/ Gynaecology	2 (13.30	13 (86.7)	15
Oesophageal	1 (33.3)	2 (66.7)	3
Open reduction	4 (50.00	4 (50.0)	8
Orchidectomy	4 (33.3)	8 (66.7)	12
Orthopaedic	10 (35.7)	18 (64.3)	28
Paediatrics	8 (30.8)	18 (69.2)	26
Prostatectomy	5 (22.7)	17 (77.3)	22
Thoracic surgery	0 (0)	8(100)	8
Thyroidectomy	-	-	0
Total	**112**	**208**	**320**

X^2 = 34.07 df = 8 P< 0.05; SB: Significant bacteriuria, NSB: Non Significant bacteriuria

Table 5: Antibiotypes of Bacterial Isolates from Indicator of Surgery and Wounds of Patients from Lagos University Teaching Hospital

Organisms	Antibiogram	No of isolates from endogenous indicator with similar clusters	No of isolates from Incision with similar clusters	Plasmid sizes (Kb)
***Klebsiella pneumoniae* (18)**	ApGnNaNtCt ApGNNaSt	4 3	6 5	52.5, 45.0
***Enterobacter sakazakii* (20)**	ApNaCtTt ApCtGnNaNt ApNaNtCr	2 3 1	5 6 3	42.5, 33.2, 27, 25.7
***Escherichia coli* (20)**	ApGnNaSt ApCtNaTtCm	3 4	7 6	42.5, 40.0, 32.8
***Acinetobacter baumanii* (15)**	ApGnNaNt ApGnNtTt	4 1	8 2	32.5, 30.0, 28.5
***Pseudomonas aeruginosa* (15)**	ApCtNaNtSt ApClCm	2 2	7 4	52.51, 50.0, 42.4
***Staphylococcus aureus* (12)**	ApChCxErPn ApCxErPn	2 2	5 3	40.5, 35.5, 30.2

Abbreviation: Ap – Ampicillin, Gn – Gentamicin, Na – Nalidixic acid, Nt – Nitrofurantoin, Ct – Cotrimoxazole, Ch, Chloramphenical, St – Streptomycin, Cr – Ceftriaxone, Cm- Cefuroxime, Er – Erythromycin, Pn – Penicillin, Cl – Colistin, Cx - Cloxacillin

DISCUSSION

Endogenous route has been shown to be a major source of nosocomial infection. This study proved surgical procedures as risk factors. Isolation of *Enterobacter cloacae*, *Proteus sp*, and *Pseudomonas aeruginosa* from segmented colon and their subsequent isolation from wounds indicated that they were from endogenous source. Such isolations confirm reason for selective decontamination of the digestive tract prior to abdominal surgery (CDC, 1991; Kurado, 1993; Nichols, 2004). The presence of pathogens on tissues and in organ/space with their subsequent isolation from wound implicates surgical manipulations. Ordinarily, activities of such organisms are passive in the colon, but surgical intervention may aid in their displacement leading to their role as opportunistic pathogens.

Isolation of *Pseudomonas sp* was twice that of *Escherichia coli*, indicating break in intact mucous membrane. Furthermore infections were dependent on the immune status of the patients and the infection rates were statistically significant. Most of nosocomial infections occurred after clean contaminated and contaminated procedures. This finding is also consistent with prevention reports (Horan *et al*., 1993; Jacob *et al*., 2000). The infection rate in the ward was 43.1% while the theatre was 41.9%, the difference was statistically significant. *Pseudomonas aeruginosa* was the most commonly isolated pathogens from theatre and on the ward while *Staphylococcus aureus*, *E. coli* and coagulase negative *Staphylococcus* were commonly isolated from patients recovering in the wards. Patients who were septic before coming to hospital had more complications from infection as observed in this study, also in similar studies (Avery *et al.,* 2006; Isibor *et al.,* 2008). Isolates from pus obtained during surgery were mainly *Enterobacter sp* and *Pseudomonas sp.* The high incidence of septic shock from *Enterobacter sp*. has been emphasised (Tubinstein *et al*., 1993). This calls for further study on this pathogen.

Isolation of *Streptococcus sp* from urology patients may serve as clue to certain internal damage. The organism produces hyaluronidase, an enzyme known to hydrolyse hyaluronic acid - the cementing substance of tissue. It is also known to destroy skin graft (Krizek and Robson, 1967). This calls for laboratory investigation and clearing of infection before further therapeutic options (Anguzu and Olila, 2007). The overall significant bacteriuria was almost twice that of non-significant bacteriuria. Analysis of catheter-associated urinary tract infection showed that higher rates were recorded among patients in the ward as days increased. Bacterial pathogens are well-known to complicate catheter associated infections (Burke *et al*., 1981; Onche and Adedeji, 2004). In this study, bacterial pathogens isolated from patients in theatre were also related to the ward. Besides, patients with

genitourinary manipulations had higher rates thus emphasising the need to remove urinary catheters as early as possible.

In this study, it was found that most of the isolates were resistant to more than three antibiotics. Such multidrug resistance is most likely due to indiscriminate use of antibiotics especially in surgical procedures. There was also noticeable relationship between the antibiotic resistance patterns and plasmid patterns generated. Easily transferable multiple drug resistant genes are carried by the different plasmids through conjugation (Alam *et al.,* 2010). The use of other molecular biology tools should provide more information on the spread of antibiotic resistant bacteria in the hospital setting.

Evidently, findings from this study proved that organisms isolated from endogenous indicators of surgery were found on incisions. They were linked to organisms causing intrinsic infections in patients (Kropec *et al*, 1993; Dhars *et al*., 2007). The isolation of enterobacteriaceae with a predominance of *Klebsiella pneumoniae* found in wound of colon surgery and frequent isolation of *Pseudomonas sp* from orthopaedic wound and catheter-associated urinary tract infections, were indicators for treatments of patients. Feedback from surgeons showed that patients got better and reductions in the number of hospital days were achieved. Factors responsible for endogenous organisms infecting surgical patients were corrected and some were prevented. These include meticulous pre-operative preparation of surgical patients to reduce microbiologic burden of patients' bowels, respiratory tract, genital tract etc, proper wound dressing and barrier precautions during catheter insertion in neutropenic and high risk patients. Therefore, feedback to surgeons, amendment in some surgical techniques, culture and sensitivity, appropriate use of antibiotics and correction of underlying derangement are essential in the management of post-surgical sepsis.

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