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Antibiotic resistance profile of bacteria isolated from patients admitted with postoperative infection in a regional hospital center in Togo

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The treatment of postoperative infections is becoming difficult because of the increasing resistance rate to antibiotics. This study aimed to identify postoperative bacterial infections and determine their current antimicrobial resistance to commonly prescribed antibiotics drugs. A cross sectional study was carried out on patients operated upon and followed up for development of infection on the surgical site until the time of discharge. On 36 cases analyzed prospectively, wound swab samples were collected and processed for bacterial isolation and antimicrobial susceptibility. A total of 35 bacterial pathogens were identified from 35 cases. *Klebsiella* and *Staphylococcus* were the leading isolates accounting to 28% each, followed by *Escherichia coli* (14%) and *Citrobacter* (9%), *Pseudomonas* and *Proteus* (6% each), *Enterobacter*, *Yersinia* and *Acinetobacter* (3% each). The highest resistance rate (90%) of *Klebsiella* was to amoxicillin-clavulanic acid, cefalotin, cefazolin, cefoxitin followed by ciprofloxacin and the lowest resistance rate (10%) was to aztreonam and to all macrolides used. The highest resistance rate (100%) of *Staphylococcus* was to penicillin. *Klebsiella* and *Staphylococcus* were the most common causative agents of postoperative infections. The multidrug resistance level of these bacteria isolated was very alarming so that any empirical prophylaxis and treatment need careful selection of effective drugs.

Key words: Antibiotic, resistance, bacteria, postoperative infection.

INTRODUCTION

Postoperative infections become more and more frequent in hospitals and a major public health problem worldwide (Auajjar et al., 2006; Simon et al., 2007). They constitute the major causes of morbidity and mortality, functional disability, emotional suffering and economic burden among the hospitalized patients (Daurat et al., 2000; Priano et al., 2005). They also result in greater lengths of stay in hospital and additional costs (Dembele, 2005; Han et al., 2012). Postoperative wound infection can occur from first day onwards to many years after an operation but commonly occurs between the fifth and tenth days after surgery. It may originate during the operation from sources in the ward or as a result of some complications (Dembele, 2005).

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Most postoperative wound infections are hospital acquired and vary from one hospital to the other and within a given hospitals. The common bacterial pathogens present in the wound swab are *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Yersinia* spp., *Proteus* spp., *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus* spp., *Pseudomonas* spp. and *Acinetobacter* spp. As a result of extensive uses of antimicrobial agents, hospital-acquired infections pathogens have shifted away from easily treatable bacteria towards more resistant bacteria. This change is causing a very important problem for post-operative infection control and its prevention in developing countries owing to poor infection program, crowding hospital environment and irrational prescription of antimicrobial agents (Rayes et al., 2013).

Therefore, the aim of this study was to isolate bacterial pathogens from hospital acquired surgical site infection and determine their current antimicrobial resistant patterns among patients who were admitted in surgical and gynecology wards.

MATERIALS AND METHODS

Subjects

This prospective study was carried out from January 2011 to December 2012 in Tsevie, a regional hospital center in Togo. During the study, all patients admitted in surgical and genecology wards with postoperative infection, more than five days after surgical operation, were included. Clinical examination was conducted by physician to exclude those who were not in these conditions. Each patient accepted to take part in this study.

Sample collection and bacterial identification

A predesigned and structure questionnaire was developed and used to collect patient's data on socio-demographic characteristics. During the study period a total of 36 patients admitted in surgical and genecology wards were enrolled and two wound specimens per patient were collected aseptically using cotton swabs by experienced laboratory personnel and the swabs were immediately dipped into a sterile tube containing two drops of sterile normal saline delivered to the laboratory within five minutes of collection. Then, one of the wound swabs was sowed on cooked Blood agar with isovitalex under CO_2, Blood agar with Nalidixic acid, Bromocresol Pourpre agar, Eosin Methylene Blue agar or Drigalski agar, Mannitol Salt Agar, Thioglucolate broth media plates (all Oxoid, Ltd, England). The inoculated agar plates were incubated into incubator INCOUCELL Ref MMM Med center Einrich tungel Gmbh MMM- Group at 37°C overnight. The other wound swab was used for Gram staining smears to make presumptive diagnosis on microscope. Identification of cultured isolates was done after a biochemical gallery which showed their characters (Clave et al., 2009).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by Kirby-Bauer's disk diffusion method on Muller-Hinton agar in accordance with the standards of the Clinical Laboratory Standards Institute guidelines (Bauer et al., 1966). Obviously, a loop full of bacteria was taken from a pure culture colony and was transferred to a tube containing 5 ml of phosphate buffer saline and mixed gently until it formed a homogenous suspension and the turbidity of the suspension was adjusted to the turbidity of McFarland 0.5 standard in a tube. For each isolated bacteria, the standardized inoculums were inoculated on Mueller Hinton antibiotic sensibility medium (Oxoid, Ltd, England). The plates were incubated at 37°C for 18-24 h and the interpretation of the results of the antimicrobial susceptibility was based on the CLSI criteria as sensitive, intermediate and resistant by measuring diameter of the inhibition zone (Clave et al., 2009). All intermediate readings were taken as resistant during data entry. The antibiotic concentration per disk was as follows: penicillin (PEN) 10 units, ampicillin (AMP) 10 µg, amoxicillin (AMX) 20 µg, amoxicillin + clavulanic acid (AMC) 20 µ/10 µg, carbenicillin (CAR) 100 µg, oxacillin (OXA) 5 µg, piperacillin (PIP) 75 µg, piperacillin + tazobactam (IZP) 75 µg/10 µg, ticarcillin (TIC) 75 µg, imipenem (IPM) 10 µg, cefalotin (CF) 30 µg, cefalexin (CN) 30 µg, cefazolin (CFZ) 30 µg, cefoxitin (FOX) 30 µg, cefotaxim (CTX) 30 µg, ceftriaxone (CRO) 30 µg, aztreonam (ATM) 20 µg, cefuroxime (CXM), ceftazidime (CAZ) 30 µg, cefoperazone (CFP) 75 µg, cefixime (CFM) 10 µg, netromicin (NET) 30 µg, gentamicin (GEN) 15 µg, amikacin (AN) 30 µg, tobramycin (TM) 10 µg, kanamycin (KAN) 30 µg, sisomicin (SIS) 30 µg, tetracycline (TET) 30 µg, oxytetracycline (OXT) 30 µg, doxycycline (DOT) 30 µg, minocycline (MIN) 30 µg, erythromycin (ERY) 15 µg, spiramycin (SPI) 15 µg, lincomycin (LIN) 15 µg, josamycin (JOS) 15 µg, virginamycin, (VIR) 15 µg, pristinamycin (PRI) 15 µg, oleandomycin (OLE) 15 µg, chloramphenicol (CMP) 30 µg, thiamphenicol (THI) 30 µg, colistin (COL) 25 µg, trimethoprime-sulfamide (SXT) 25 µg, nalidixic acid (NAL) 30 µg, pipemidic acid (PI) 5 µg, ofloxacin (OFX) 5 µg, norfloxacin (NOR) 5 µg, ciprofloxacin (CIP) 5 µg.

Statistical analysis

Data entry and statistical analysis were performed using Epi info version 6.04-c fr software. Comparisons were made using Pearson Chi-square and Student t-test. P-value strictly lower than $p<0.05$ was considered indicative of a statistically significant difference.

RESULTS

A total of 36 samples were analyzed. Among them, 23 (64%) were obtained from males and 13 (36%) from females. *Klebsiella* spp. (28%) and *Staphylococcus* spp. (28%) were the most frequent microorganisms isolated from the wound swabs followed by *Escherichia coli* (14%), *Citrobacter* spp. (9%), *Proteus* spp., *Pseudomonas* spp. (6% each), *Enterobacter* spp., *Yersinia* spp. and *Acinetobacter* spp. (3% each) (Table 1).

The results of the resistance rates to different antibiotics are shown in Tables 2, 3, 4, 5, 6, 7 and 8. The antimicrobial drug resistance profile of isolated bacteria showed that the highest rate of resistance of *Klebsiella* spp. was to amoxicillin+clavulanic acid, cefalotin, cefazolin, cefoxitin, trimethoprime-sulfamide, ofloxacin, norfloxacin and nalidixic acid (90% each), followed by ciprofloxacin, cefotaxime, ceftriaxone cefuroxime, tetra-cycline and doxycycline (80%). The lowest rate of resistance was to aztreonam and every macrolide (10%). *Citrobacter* spp. was highly resistant to penicillin G, ampicillin, amoxicillin + clavulanic acid carbenicillin, oxacillin, ticarcillin, cefalotine, cefazolin, cefoxitin, cefuroxime, ofloxacin,

Table 1. Isolated strains and percentage.

Isolated strains		Number	Pourcentage (%)
Enterobacteria	*Klebsiella* spp.	10	28
	Citrobacter spp.	03	09
	Enterobacter spp.	01	03
	Yersinia spp.	01	03
	Proteus spp.	02	06
	Escherichia coli	05	14
Gram positive cocci	*Staphylococcus* spp.	10	28
Other gram negative bacilli	*Pseudomonas* spp.	02	06
	Acinetobacter spp.	01	03

Table 2. Resistance of bacterial isolates to penicillin.

Strains	Antibiotics										
	PEN	AMP	AMX	AMC	CAR	OXA	TIC	PIP	IZP	IMP	Arithmetic average
Klebsiella spp.	6	7	4	9	6	5	5	4	4	4	
	60%	70%	40%	90%	60%	50%	50%	40%	40%	40%	54%
Citrobacter spp.	3	3	0	3	3	3	3	0	0	0	
	100%	100%	0%	100%	100%	100%	100%	0%	0%	0%	60%
Enterobacter spp.	0	1	0	1	0	1	0	0	0	0	
	0%	100%	0%	100%	0%	100%	0%	0%	0%	0%	30%
Yersinia spp.	0	1	0	1	1	0	0	0	0	0	
	0%	100%	0%	100%	100%	0%	0%	0%	0%	0%	30%
Proteus spp.	0	2	0	2	1	0	0	0	0	0	
	0%	100%	0%	100%	50%	0%	0%	0%	0%	0%	25%
Escherichia coli	3	4	0	4	3	2	3	1	0	0	
	60%	80%	0%	80%	60%	40%	60%	20%	0%	0%	40%
Staphylococcus spp.	10	9	6	1	6	1	1	1	1	1	
	100%	90%	60%	10%	60%	10%	10%	10%	10%	10%	37%
Pseudomonas spp.	1	1	1	1	2	1	2	0	0	0	
	50%	50%	50%	50%	100%	50%	100%	0%	0%	0%	45%
Acinetobacter spp.	0	0	0	1	0	0	0	0	0	0	
	0%	0%	0%	100%	0%	0%	0%	0%	0%	0%	10%
Arithmetic Average	41.1%	76.6%	16.6%	81.1%	58.8%	38.8%	35.5%	07.7%	05.5%	05.5%	

norfloxacin, ciprofloxacin, trimethoprime - sulfamide, all the cyclines and macrolides used in this study (100%). The highest resistance rate (100%) of *Enterobacter* spp. was to ampicillin, amoxicillin + clavulanic acid, oxacillin, cefalotin, cefazolin, chloramphenicol, thiamphenicol and trimethoprime-sulfamide. *Yersinia* spp. was highly resistant (100%) to ampicillin, amoxicillin + clavulanic acid, carbenicillin, cefalotin, cefazolin, tetracycline, doxycycline, trimethoprime-sulfamide, ofloxacin, norfloxacin and ciprofloxacin. *Proteus* spp. also showed resistance to ampicillin, amoxicillin + clavulanic acid, chloramphenicol, thiamphenicol, trimethoprime-sulfamide, almost (100%) the cephalosporins used except cefalexin, aztreonam and cefoperazone. The highest rate of resistance (100%) of *Escherichia coli* was to trimethoprime-sulphamide, ofloxacin, norfloxacin and ciprofloxacin, followed by ampicillin, amoxicillin + clavulanic acid, netromicin, gentamicin, kanamycin, sisomicin, tetracycline, doxycycline, nalidxic acid and pipemidic acid (80%). Penicillin G, ampicillin and tetracycline were drugs which showed high rate of resistance to *Staphylococcus* spp. (respectively 100, 90 and 80%). *Pseudomonas* spp. was highly resistant (100%) to carbenicillin, ticarcillin, cefotaxime, netromicin, kanamycin, tetracycline, doxycycline, ofloxacin,

Table 3. Resistance of bacterial isolates to cephalosporins.

Strains	Antibiotics											
	CF	**CN**	**CFZ**	**FOX**	**CTX**	**CRO**	**ATM**	**CXM**	**CFP**	**CAZ**	**CFM**	**Arithmetic Average**
Klebsiella spp.	9 90%	4 40%	9 90%	9 90%	8 80%	8 80%	1 10%	8 80%	2 20%	4 40%	2 20%	58.2%
Citrobacter spp.	3 100%	0 0%	3 100%	3 100%	2 67%	2 67%	0 0%	3 100%	2 67%	2 67%	2 67%	66.8%
Enterobacter spp.	1 100%	0 0%	1 100%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	18.2%
Yersinia spp.	1 100%	0 0%	1 100%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	18.2%
Proteus spp.	2 100%	0 0%	2 100%	2 100%	2 100%	2 100%	0 0%	2 100%	1 50%	2 100%	2 100%	77.3%
Escherichia coli	2 40%	0 0%	2 40%	2 40%	0 0%	0 0%	0 0%	2 40%	0 0%	0 0%	0 0%	14.5%
Staphylococcus spp.	1 10%	0 0%	1 10%	2 20%	1 10%	1 10%	0 0%	1 10%	1 10%	1 10%	1 10%	09.1%
Pseudomonas spp.	1 50%	1 50%	1 50%	1 50%	2 100%	1 50%	0 0%	0 0%	1 50%	0 0%	0 0%	36.4%
Acinetobacter spp.	1 100%	1 100%	1 100%	1 100%	1 100%	1 100%	0 0%	0 0%	0 0%	0 0%	0 0%	54.5%
Arithmetic Average	76.6%	21.1%	76..6%	55.5%	50.7%	45.2%	01.1%	36.6%	21.9%	24.1%	21.1%	

Table 4. Resistance of bacterial isolates to aminosides.

Strains	Antibiotics						
	NET	**GEN**	**AN**	**TM**	**KAN**	**SIS**	**Arithmetic Average**
Klebsiella spp.	5 50%	6 60%	3 30%	5 50%	4 40%	6 60%	48.3%
Citrobacter spp.	3 100%	3 100%	3 100%	3 100%	3 100%	3 100%	100%
Enterobacter spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0%
Yersinia spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0%
Proteus spp.	1 50%	1 50%	0 0%	0 0%	0 0%	1 50%	25%
Escherichia coli	4 80%	4 80%	2 40%	3 60%	4 80%	4 80%	70%
Staphylococcus spp.	3 30%	3 30%	2 20%	3 30%	4 40%	2 20%	28.3%
Pseudomonas spp.	2 100%	1 50%	0 0%	0 0%	2 100%	1 50%	50%
Acinetobacter spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0%
Arithmetic Average	45.5%	41.1%	21.1%	26.6%	40%	40%	

norfloxacin and ciprofloxacin. Similarly, *Acinetobacter* spp. showed 100% resistance to amoxicillin + clavulanic acid, cefalotin, cephalexin, cefazolin, ceftriaxone, cefoxitin, cefotaxime, all the cyclines used, chloramphenicol, thiamphenicol, trimethoprime-sulfamide and all the quinolones used in this study.

Table 5. Resistance of bacterial isolates to tetracyclines.

Strains	Antibiotics				
	TET	OXT	DOT	MIN	Arithmetic Average
Klebsiella spp.	8 80%	7 70%	8 80%	7 70%	75%
Citrobacter spp.	3 100%	3 100%	3 100%	3 100%	100%
Enterobacter spp.	0 0%	0 0%	0 0%	0 0%	00%
Yersinia spp.	1 100%	0 0%	1 100%	0 0%	50%
Proteus spp.	0 0%	0 0%	0 0%	0 0%	00%
Escherichia coli	4 80%	2 40%	4 80%	2 40%	60%
Staphylococcus spp.	8 80%	5 50%	7 70%	5 50%	62.5%
Pseudomonas spp.	2 100%	1 50%	2 100%	1 50%	75%
Acinetobacter spp.	1 100%	1 100%	1 100%	1 100%	100%
Arithmetic Average	71.1%	45.5%	70%	45.5%	

Table 6. Resistance of bacterial isolates to macrolides.

Strains	Antibiotics							
	ERY	SPI	LIN	JOS	VIR	PRI	OLE	Arithmetic Average
Klebsiella spp.	1 10%	1 10%	1 10%	1 10%	1 10%	1 10%	1 10%	10%
Citrobacter spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	00%
Enterobacter spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	00%
Yersinia spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	00%
Proteus spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	00%
Escherichia coli	3 60%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	08.57%
Staphylococcus spp.	3 30%	0 0%	4 40%	0 0%	0 0%	2 20%	0 0%	12 .85%
Pseudomonas spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	00%
Acinetobacter spp.	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	00%
Arithmetic Average	11.1%	01.10%	05.6%	01.1%	01.1%	01.1%	01.1%	

DISCUSSION

This study shows the distribution of antibiotic resistance pattern of bacterial species isolated from patients with postoperative infection in a regional hospital center, Togo. It revealed that 35 (97.22%) out of 36 wound

Table 7. Resistance of bacterial isolates to phenicoles, sulfamides and apparents.

Strains	Antibiotics				
	CMP	THI	COL	SXT	Arithmetic Average
Klebsiella spp.	5 50%	5 50%	0 0%	9 90%	47.5%
Citrobacter spp.	2 67%	2 67%	0 0%	3 100%	58.5%
Enterobacter spp.	1 100%	1 100%	0 0%	1 100%	75%
Yersinia spp.	0 0%	0 0%	0 0%	1 100%	25%
Proteus spp.	2 100%	2 100%	0 0%	2 100%	75%
Escherichia coli	2 40%	2 40%	0 0%	5 100%	45%
Staphylococcus spp.	5 50%	5 50%	0 0%	2 20%	30%
Pseudomonas spp.	1 50%	1 50%	0 0%	1 50%	37.5%
Acinetobacter spp.	1 100%	1 100%	0 0%	1 100%	75%
Arithmetic Average	61.8%	61 .8%	0%	84.4%	

Table 8. Resistance of bacterial isolates to quinolones.

Strains	Antibiotics					
	OFX	NOR	CIP	NAL	PI	Arithmetic average
Klebsiella spp.	9 90%	9 90%	8 80%	9 90%	5 50%	80%
Citrobacter spp.	3 100%	3 100%	3 100%	2 67%	0 0%	73.4%
Enterobacter spp.	0 0%	0 0%	0 0%	0 0%	0 0%	00%
Yersinia spp.	1 100%	1 100%	1 100%	0 0%	0 0%	60%
Proteus spp.	1 50%	1 50%	1 50%	1 50%	1 50%	50%
Escherichia coli	5 100%	5 100%	5 100%	4 80%	4 80%	92%
Staphylococcus spp.	4 40%	4 40%	5 50%	1 10%	1 10%	30%
Pseudomonas spp.	2 100%	2 100%	2 100%	1 50%	1 50%	80%
Acinetobacter spp.	1 100%	1 100%	1 100%	1 100%	1 100%	100%
Arithmetic Average	75.5%	75.5%	75.5%	49.6%	37.7%	

swabs samples which were obtained from postoperative infection suspected patients were positive. Of the 36 patients, 13 (35.1%) were females and 23 (63.9%) were males. Similar findings have been observed by Togo et al. (2009) in Bamako. Other findings observed by Dembele (2005) in surgery and pediatric service of

GABRIEL TOURE hospital are different from ours. *Klebsiella* spp. (28%) and *Staphylococcus* spp. (28%) were the most prevalent microorganisms isolated from the wound swabs. These results are similar to those observed by Arias et al. (2003) and Zaidi et al. (2005) which revealed that *E. coli* was the most prevalent microorganism isolated. The isolated Gram-negative bacilli's rate of resistance to penicillin was average (36.75%). *Citrobacter* spp. were the most resistant (60%) among them. The rate of resistance of the isolated germs to cephalosporin varies between 9.1% and 77.3%. Similar findings have been observed by Dembele (2005) and Timbine et al. (1997) in Bamako hospital (between 0 and 67%). This study has revealed that the resistance rate of the isolated microorganisms against aminosides varies between 0 and 100%. The results found by Dagnra et al. (2001) in Lome hospital on *Staphylococcus* to gentamicin (19.8%) were below ours (30%). This difference may probably have its origin in the regularity of the prescription of this drug. The resistance rate of the isolated germs tetracycline varies between 0 and 100%. The results found by Timbine et al. (1997) on Gram-negative bacilli varied between 75 and 96.5%. Their findings are similar to the results of this study. In contrast to the other drugs used in this study, all the isolated microorganisms were sensible to macrolides. The rate resistance of these germs against these drugs varies between 0 and 12.85%. Different findings were observed by Moyen et al. (2007) on *Staphylococcus* (71.79%). This difference may also probably have its origin in the regularity of prescription of these drugs in the respective sites. This study has revealed that the rate of the isolated germs against phenicoles, sulfamides and apparent varies between 25 and 75%. The results by Moyen et al. (2007) about *Staphylococcus* (81.75%), are above these values. The resistance rate of the isolated bacteria to quinolones varies between 0 and 100%. Similar findings were observed by Dembele (2005) in Bamako (77.5%). The results found by Moyen et al. (2007) on *Staphylococcus* (51.28%) are above our values (30%).

The resistance rate of germs against antibiotic is determined by the interactions of several factors such as primary disease and its severity, duration of hospitalization and treatment, and invasive interventions. Moreover, the incidence of post-operative wound infections has been increasing and its treatment has become more complicated because of the pathogens with increasing resistance to antibiotics (Anagonou et al., 1994). Limitation of the study is that anaerobic bacteria profile and fungal cultures were not done on wound swabs obtained from post-operative wound infection. A continuous monitoring and update studies on the local microbial isolates are an essential and mandatory requirement for a better management and treatment of post-operative wound infections. This would be supplemented with proper infection prevention and control measures and a sound antibiotic policy. This would result in better patient care, safety and health care outcomes.

In conclusion, enteric bacteria were more frequently involved in postoperative infection in Tsevie, a regional hospital center of Togo. *Klebsiella* spp. and *Staphylococcus* spp. were the most predominant. The rate resistance of the isolated bacteria to commonly available and prescribed antimicrobial drugs varied from each family and the rhythm of prescription of antibiotics in the hospital center. The hospital center needs to make a concerted effort to minimize hospital acquired infection by following strict aseptic operation procedures, effective method of sterilezation and patient management. At last, the appropriate use of antibiotics according to the standard antimicrobial susceptibility test is proposed to reduce the prevalence of postoperative infections and resolve this problem of antibiotic resistance of bacteria.

Conflict of interest

The author(s) have not declared any conflict of interests.

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