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BACTERIAL PATHOGENS ASSOCIATED WITH SECONDARY PERITONITIS IN LAGOS UNIVERSITY TEACHING HOSPITAL (LUTH)

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ABSTRACT
Secondary peritonitis is a common and serious form of intra-abdominal infection, often associated with high morbidity and mortality. The overall patient outcome has not markedly improved in spite of advances in patient management. There is therefore need to study the pattern of bacterial pathogens associated with secondary peritonitis in Lagos University Teaching Hospital (LUTH) and the antibiotic susceptibility pattern as that would help in formulation of empiric antibiotic policy on peritonitis and improve the outcome/prognosis of the patients.

A prospective study of 35 patients with suspected peritonitis at LUTH between February, 2002 and June 2003 was done. Peritoneal fluids of these patients were collected intra-operatively under aseptic conditions. The specimens were subjected to aerobic and anaerobic studies.

Twenty-seven isolates were identified aerobically with *Escherichia coli* being the most predominant organism 11(31.4%) followed by *Staphylococcus aureus* 6(17.1%) then *Klebsiella* spp 4(11.4%). Anaerobic culture showed *Prevotella* species as the most predominant 14(40%) followed by *Bacteroides fragilis* group 8(22.9%). Overall, *Prevotella* species were the most predominant organisms isolated in LUTH patients with secondary peritonitis.

Key words: Bacterial Pathogens, Secondary Peritonitis, Lagos

INTRODUCTION
Peritonitis is defined as inflammation of the serosal membrane that lines the abdominal cavity and the organs contained therein[1],it is often caused by introduction of an infection into the otherwise sterile peritoneal environment through perforation of the bowel, such as ruptured appendix or colonic diverticulum. The disease may also be caused by introduction of a chemically irritating material such as gastric acid from a perforated gall bladder or a lacerated liver. In general, the term peritonitis refers to a constellation of signs and symptoms, which includes abdominal pain and tenderness on palpation, abdominal wall muscle rigidity and systemic signs of inflammation.

Peritoneal infections are classified as primary (i.e. spontaneous), secondary (i.e. related to a pathologic process in a visceral organ) or tertiary (i.e. persistent or recurrent infection after adequate initial therapy). The most common etiology of primary peritonitis is spontaneous bacteria peritonitis (SBP) due to chronic liver disease [2,3]. The prevalence in children apparently has been decreasing due to widespread use of antibiotics for minor upper respiratory tract illnesses[4]. The microbiological yield of primary peritonitis is usually a pure growth of organisms like *Escherichia coli* (*E.coli*), *Streptococcus pneumoniae* and Group A beta hemolytic streptococcus [4,5,6].

The common etiologic entities of secondary peritonitis (SP) include perforated appendicitis, perforated gastric and duodenal ulcer disease, perforated (sigmoid) colon caused by diverticulitis, volvulus or cancer and strangulation of the small bowel[7]. It could also be caused by traumatic perforation associated with typhoid, tuberculosis[8,9], amebic, *Strongyloides*, *Cytomegalovirus*, ulcers in immunocompromised persons, appendicitis, bile peritonitis, pancreatitis and septic abortion. Infrequently secondary peritonitis is caused by exogenous micro-organisms such as *Staphylococcus aureus* and *Neisseria gonorrhoea* which cause infection intra-abdominal or adjacent viscera and spread to the peritoneum. Secondary peritonitis is typically polymicrobial and the pathogens in most cases are derived from the gastro-intestinal tract. The facultative micro-organisms are *E.coli, Klebsiella, Enterobacter, Proteus* species and *Enterococci*.

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Anaerobes account for well over 99% of the normal colonic flora and accordingly, are frequently seen in intra-abdominal infections[10]. The obligate anaerobes usually seen are *Bactericides fragilis*, p*melaninogenica, Peptococcus,Peptostreptococcus, Fusobacterium, Eubacterium lentum* and *Clostridium* spp. Other less commonly pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida*[11]..

The study was done to determine the bacterial pathogens associated with secondary peritonitis in Lagos University Teaching Hospital (LUTH) Idi-Araba, Lagos, Nigeria.

MATERIALS AND METHODS

Study population

The study group involved all the patients with suspected secondary peritonitis who presented at the Lagos University Teaching Hospital (LUTH) Idi-Araba between February 2002 and June 2003. A total of 35 patients were seen.

Ethical Issues

The research posed no risk to the subjects and added no extra cost to them. Approval for the study was obtained from the Research and Ethics Committee of LUTH.

Media used: Blood agar base and Mac Conkey agar were used for aerobic culture while Chocolate agar, Bacteroides Bile Eesculin agar (BBE) and Neomycin-Vancomycin laked Blood agar (NVLB) were used for anaerobic culture. BBE agar was for selection of the *Bacteroides fragilis* group[12] and *Bilophila*[13], while NVLB or kanamycin-vancomycin laked Blood agar for selection of pigmented and other *Prevotella* species and *Bacteroides*[14].

METHODOLOGY

Cases of suspected secondary peritonitis were followed up from the Accident and Emergency center until the diagnosis was confirmed at laparatomy.

Specimen collection and processing.

Intra-operatively, 10 mls of peritoneal fluid was collected asceptically. This was quickly examined macroscopically for color, odor and purulence. The specimen was inoculated onto plates for aerobic and anaerobic cultures. Robertson's cooked meat medium was used for back-up culture.

Microscopy: Initial gram stain of specimen was done to determine the characteristic cell morphology, types and relative number of microbes and host cells present. Smears were fixed for 30 seconds in methanol to preserve red and white blood cell morphology. Basic fuchsin 0.5% was used as counter stain to enhance staining of gram negative anaerobes. Aerobic culture plates were incubated at 37°C for 24hrs, while anaerobic culture plates were incubated up to 7 days before being discarded as negative. Inoculum in cooked meat medium was observed for 7 days before being discarded as negative. When there was evidence of growth, the specimen was gram stained, sub cultured unto Blood agar and incubated aerobically. It was also sub cultured unto Chocolate agar, NVLB agar and BBE agar and incubated anaerobically.

Pseudomonas aeruginosa was used as biological indicator for anaerobic incubation and Palladium coated alumina pellets were used as catalysts..

Identification

Aerobes: Isolates were identified based on their colonial morphology, pigmentation, haemolysis, Gram stain reactions, Oxidase, Citrate utilization, Coagulase, Catalase, Methyl-red/Voges-Proskauer (VP) reactions, Motility,Indole, Urea, Hydrogen Sulphide(H2S) production and fermentation of glucose and lactose.

Anaerobes: After incubation organisms were identified based on colonial morphology, presence or absence of haemolysis, Pigmentation, reactions on Gram stain, Indole, Catalase and Esculin, Motility: Fermentation of Sucrose, Maltose and Trehalose.

Susceptibility testing.

This was carried out on the aerobic isolates using standardized disc diffusion technique using Mueller-Hinton agar (Oxoid). The zone of inhibition was measured and interpreted using the National Committee for Clinical Laboratory Standard (NCCLS) guideline (2000)[15].

Isolates were tested against the following antibiotics: Ampicillin (amp), Cotrimoxazole (cot), Gentamicin (gen), Streptomycin (str), Tetracycline (tet), Ceftriaxone (cro), Cefotaxine (cxm) and Ceftazidine (caz). Control organisms used were *Escherichia coli* ATCC 25922, *E.coli* ATCC 35218, *Pseudomonas* ATCC 27853, *Staphylococcus aureus* ATCC 29213 (obtained from Smithkline Beecham Laboratory Paris France). Susceptibility testing of the anaerobes could not be done.

Data analysis.

The data was analyzed using EPI- INFO 6.02 software developed by the Centre for Diseases Control, Atlanta, Georgia.

RESULTS

Thirty-five patients with suspected secondary peritonitis were used in the study. Aerobic culture yielded 27(77.1%) isolates, while 8 samples (22.9%) had no growth. The organisms isolated were *Escherichia coli*-11, *Klebsiella* spp-4, *Proteus mirabilis* -2 *Citrobacter*-2, *Pseudomonas*-1,

Staphylococcus aureus-6. This is seen in Table 1.
Anaerobic culture yielded growth in 26 samples. Isolates were *Prevotella* spp-14(40%) *Bacteroides fragilis*-8(22.9%), *Bilophila Putridinus*-2[5.7%], *Peptostreptococcus*-2(5.7%) as seen in Table 2.
Isolation of both aerobic and anaerobic organisms from sample occured in 22 samples, 4 samples yielded only aerobic organism which was *Prevotella heparinolytica*. There was no microbial growth in 8 samples each of aerobic and anaerobic incubation. Yeast was isolated in 1 patient on aerobic and anaerobic incubation. The susceptibility pattern of the aerobic organisms to various groups of antimicrobials used was not very encouraging. Less than half of *E.coli* isolates [45.5%] were susceptible to Genticin and Ofloxcicin while only 25% of *Klebsiella* isolates were susceptible to Cefotaxine and Ceftazidine. There was 100% resistance to Ampicillin in all the isolates. *Staphylococcus aureus* isolates showed moderate susceptibility [66.7%] to Cefotaxime as seen in Table 3.

Table 1: **Distribution of Organisms on aerobic culture**

Organism	*Number Isolated N = 35*	*Percentage of total*
Escherichia coil	11	31.4
Klebsiella	4	11.4
Pseudomonas	1	2.9
Proteus mirabilis	2	5.7
Citrobacter	2	5.7
Staphylococcus aureus	6	17.1
Candida	1	2.9
No growth	8	22.9
Total	**35**	**100**

Table 2: **Distribution of Organisms on anaerob culture**

Organism	*Number*	*Percentage*
Prevotella spp	14	40
- *P. Intermedia*	6	17.1
- *P. Heparinolytica*	5	14.3
- *P. Denticola*	3	8.6
Bacteroides fragilis group	8	22.9
- *B. Fragilis*	4	11.4
- *B. Ovatus*	2	5.7
- *B.thetaiotaomicron*	1	2.9
- *B. Coagulans*	1	2.9
Bilophila putridinius	2	5.7
Peptostreptococcus	2	5.7
Yeast	1	2.9
No growth	8	22.9
Total	**35**	**100**

Table 3: **Antimicrobial Susceptibility pattern of the aerobic isolates**

Antimicrobial Agents	Organisms and percentage susceptible					
	E coli N = 11	*Klebsiella* N = 4	*Proteus* N = 2	*Citrobacter* N = 2	*Pseudomonas* N = 1	*Staph aureus* N = 6
Amplicillin	Nil	Nil	Nil	Nil	Nil	Nil
Cotrimoxazole	Nil	Nil	50%	Nil	Nil	Not tested
Gentamicin	45.5%	Nil	Nil	50	Nil	33.3%
Tetracycline	Nil	Nil	50%	Nil	Nil	33.3%
Cefotaxime	Nil	25%	25%	Nil	Nil	66.7%
Ceftazidime	27.3%	25%	Nil	Nil	Nil	33.3%
Ofloxacin	45.5%	Nil	100%	50%	Nil	Not tested
Augmentin	9.1%	Nil	100%	Nil	Nil	Not tested
Erythromycin	Not tested	Not tested	Not tested	Not tested	Not tested	Nil
Streptomycin	Not tested	Not tested	Not tested	Not tested	Not tested	33.3%

DISCUSSION

Thirty five patients with secondary peritonitis were operated upon, 15 died while 20 patients survived. Out of the 20 that survived, 14 had post operative wound infections but were treated accordingly and discharged home thereafter. The following organisms were isolated from the specimen of the 15 patients that died: Prevotella spp 6, B. Fragilis 2, Yeast spp. 2, B. Thetaiotamicron 1, and B. Ovatus 1. There was no bacterial growth in 04 patients.

The bacteria organisms associated with secondary peritonitis in LUTH were *Prevotella spp* 40%, *E coli* 31.4%, *B fragilis* gmup 22.9%, *Staplylococcus aureus* 17.1%, *Klebsiella* 11.4%, *Bilophila putridinus* and *Peptostreptococus* 5.7% each. It could be seen that *Prevotella spp* were the highest isolates obtained, followed by *E coli* and *B fragilis*. This is in line with result of previous studies outside Nigeria [16 17], in which the predominant aerobic and facultative bacteria recovered were *E. Coli* and *Klebsiella* but that of the anaerobic isolates contrasts sharply in that *B.fragilis* group was the predominant anaerobe isolated in these studies but in LUTH, *Prevotella* species were the most predominant anaerobes seen [40%] then followed by *B. Fragilis* [22.9%]. The sensitivity pattern of the gram negative aerobic bacilli to various antimicrobials used was not very high. None of the isolates showed up to 50% susceptibility to the drugs including the third generation cephalosporins. This shows an increase in resistance to the group of drugs when compared with previous work done by Odugbemi [18] where 90% of *Klebsiella pneumoniae* were found to be susceptible to the third generation cephalosporins. This may be due to production of Extended Spectrum Beta-lactamases by these organisms and more studies need to be done.

The prognosis of a patient with peritonitis depends on many factors, which include the age of the patient, co-morbid conditions, the duration of peritoneal contamination, the presence of foreign material (bile or pancreatic secretions, barium), the primary intra-abdominal process and the micro-organism involved[19]. Appropriate antimicrobial therapy has been shown to reduce significantly mortality among patients with bacteremic infections caused by *Bacteroidaceae* or *Enterobacteriacea* Thirty five patients with secondary peritonitis were operated upon, 15 died while 20 patients survived. Out of the 20 that survived, 14 had post operative wound infections but were treated accordingly and discharged home thereafter. The following organisms were isolated from the specimen of the 15 patients that died: Prevotella spp 6, B. Fragilis 2, Yeast spp. 2, B. Thetaiotamicron 1, and B. Ovatus 1.

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