COMPARISON OF ANTIBIOTIC RESISTANCE PATTERNS BETWEEN LABORATORIES IN ACCRA EAST, GHANA

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ABSTRACT:- *Antibiotic resistance is increasing rapidly and developing countries are the worse affected since they provide conditions and practices that support the development and spread of resistant microbes. For better health policy on antibiotic use a national surveillance program is needed to provide baseline data from different settings. This study determines the distribution of antibiotic resistant microbes in two different laboratories and compares the results using statistical methods to ascertain if there is a variation due to human factors. Patients attending two laboratories in east Accra were recruited and samples obtained from them were cultured for microbial growth. Microbes isolated were characterized and their sensitivity to different antibiotics tested. A total of 513 samples were collected from the patients who were mostly females; 68%. The cultures that were mostly infected were urine (331), wound (116), HVS (78) and ear (26). There were few cases of throat, blood, uterus cultures but were all infected. Microbial isolates common in the different laboratories included S. aureus (96), E. coli (90), Pseudomonas aeruginosa and Proteus spp.. Microbes isolated in the different laboratories were Salmonella typhi, Shigella spp. and Streptococcus pneumonia. Microbial isolates were found resistant (over 65%) to the antibiotics ampicillin (71% and 95%), cotrimoxazole (68% and 75%) and tetracycline (70% and 80%) and moderately resistant to gentamycin (29% and 23%), erythromycin (39% and 36%) and streptomycin, and sensitive to ceftazidine and minomycin. Statistically the results from the different laboratories were found to be similar hence having the same trend. The importance of incooperating a statistical method in national surveillance program to compare results from different settings is discussed.*

INTRODUCTION

Antibiotic resistance is the development of the ability in microbial strain to tolerate bactericidal doses, which under normal circumstances tend to kill the majority of microbes being applied against. Bacterial resistance is caused by chromosomal mutation, plasmids and transposons that can transfer resistance determinants in diverse microbial species faster than new drugs can be develop to fight them (1,2,3). Selection of individuals with a particular gene that confers resistance and elimination of those without the gene confers resistance(4,5).

The problem of antibiotic was recognized and reported in the 1980's where multiple resistant strains were seen. There has been reported cases of antibiotic resistance in *Streptococcus pnuemonia*, *Mycobacterium tuberculosis* and *Enterococcus feacalis*(6). The emergence of resistant strains of *Neisseria gonorhoeae*, *Shigella spp*. and *Salmonella spp*. only pushed the cost of antibiotic treatment even higher (7,8). The traditional treatment of infections with penicillin, which is safe and affordable, is fast dating to history.

There are problems associated with selecting antibiotics for treatment based on guessed work. These include bacteria becoming resistant to therapeutic imbalance, and some bacteria such as β-heamolytic streptococcus varing widely from strain to strain in their sensitivity to antibacterial chemotherapuetic agents. The selection for pathogenic antibiotic resistance strains can be prevented by properly carrying out culture and sensitivity studies to determine the most effective antibiotics to use.

Antibiotic resistance by microbes have developed in Third world countries such as Africa, South America and Asiatic countries but only little has been reported. Antibiotic resistance is a serious health concern in Ghana. Being a developing country, it has conditions and practices that promote the development and spread of pathogenic resistance to antimicrobials. In this work, a number of antibiotics are tested against some microbial strains isolated from patients in Accra metropolis to determine their resistance levels. The results from different laboratories were also compared statistically to find the relationship.

MATERIALS AND METHODS

Patients reporting for laboratory diagnosis were screened for various culture tests. Samples for nasal, throat and HVS were obtained using sterile cotton wool swab and urethra and blood cultures were done with exudates. Fluid collected was used for eye and ear culture, aspirates and washings for skin culture and stool and urine specimen for their respective cultures. Specimen after collection were cultured as soon as possible for accuracy.

Cultures

Manufacture's instructions was used to prepare and sterilize culture media. A loopful of emulsified feaces was inoculated on Xylose deoxychocolate agar, and aerobically incubated overnight. Urine was inoculated and incubated on CLED agar. Vagina, urethra and penile specimen were incubated aerobically on chocolate agar. Pus, genital and ulcer samples were incubated for 24 hours on blood or MacConkey agar and subcultured at 48°C for 72 hours. Blood specimen was aseptically introduced into brain-heart fusion culture broth and incubated at 48 hours and subcultured on chocolate or MacConkey agar. Purulent part of sputum preparation was incubated on blood agar whereas wound and throat samples were anaerobically incubated on blood agar. The API kit was used for further characterization.

Microbial identification

Cultures were examined for significant growth after incubation. Microbes were identify by morphology, colour or type of colony after Gram staining. Biochemical tests were performed on unidentifiable microbes. These tests include standard catalase test, litmus, citrate, oxidase, indole and mortility among others.

Sensitivity test

Isolated cultures were emulsified in saline solution and plated evenly on a medium. An antibiotic disc containing specific antibiotics with standardize concentration were positioned centrally on the inoculated plate. After incubation the plates were examined for areas of no growth.

RESULTS

Characteristics:

During the first four months of 2003, a total of 513 samples were collected from patients referred for various cultural tests at two private laboratories in east Accra. Most of the patients, constituting 68%, were females.

Infected cultures

Types of cultures requested for included urine, sputum, stool, throat, blood, wound and vaginal smear. Cultures with most infections were urine (331), wound (116), high vaginal swab (74), and those moderately infected were ear swab (26), urethra (14), blood (13), and the least infected were nasal and uterus with 1 each (Fig 1)

Microbial isolates

Bacteria and fungi isolated in the two laboratories were many and varied. These included *Staphylococcus aureus* (96), *Escherichia coli* (90), *Klebsiella spp* (34), *Pseudomonas aeruginosa* (21), *Proteus spp.* (13), *Streptococcus feacalis*, and Coliforms. Some microbes were only found in each laboratory; *Streptococcus pyogenes*), *Enterococci* (2), and *Salmonella typhi* (4), *Shigella* (6), *B-haemolytic streptococcus* (1) , *Streptococcus pnuemonia* (1) and *Citrobacter* (32) (Fig 2a & 2b).

Antibiotic tests

Eighteen different antibiotics were tested for microbial sensitivity and 13 in each laboratory. Six similar antibiotics, of the same make and brand, were used in each of the two laboratories. The number of cases tested on each antibiotic ranged from 269 to 71 and 206 to 97 in the different laboratories. Microbial isolates were found to be highly resistant, over 65%, to the antibiotics ampicillin (71% and 95%), cotrimoxazole (68% and 75%), and tetracycline (70% and 80%), and moderately resistant, 25%-65%, to gentamycin (29% and 23%), erythromycin (39% and 36%), and streptomycin (36%). The antibiotics taravid (9% and 19%), ceftazidine (10%) and minocillin (16%) were found to be highly sensitive to microbes (Fig 3a & 3b). The antibiotics clarithromycin (77%) and flucoxacillin (91.3%) used in the different laboratories were highly resistant to isolates tested. Though the antibiotic Taravid was sensitive in both laboratories, there was slight differences in level of resistance between the laboratories; 19% and 5% respectively.
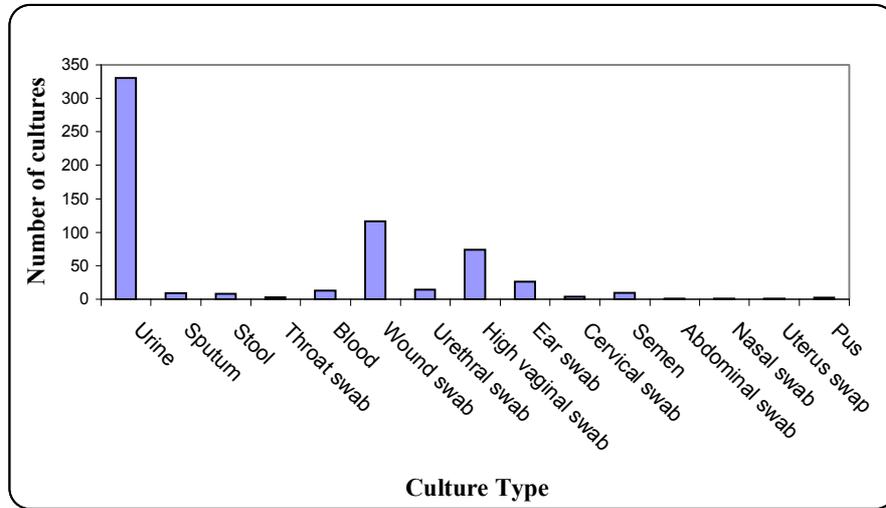
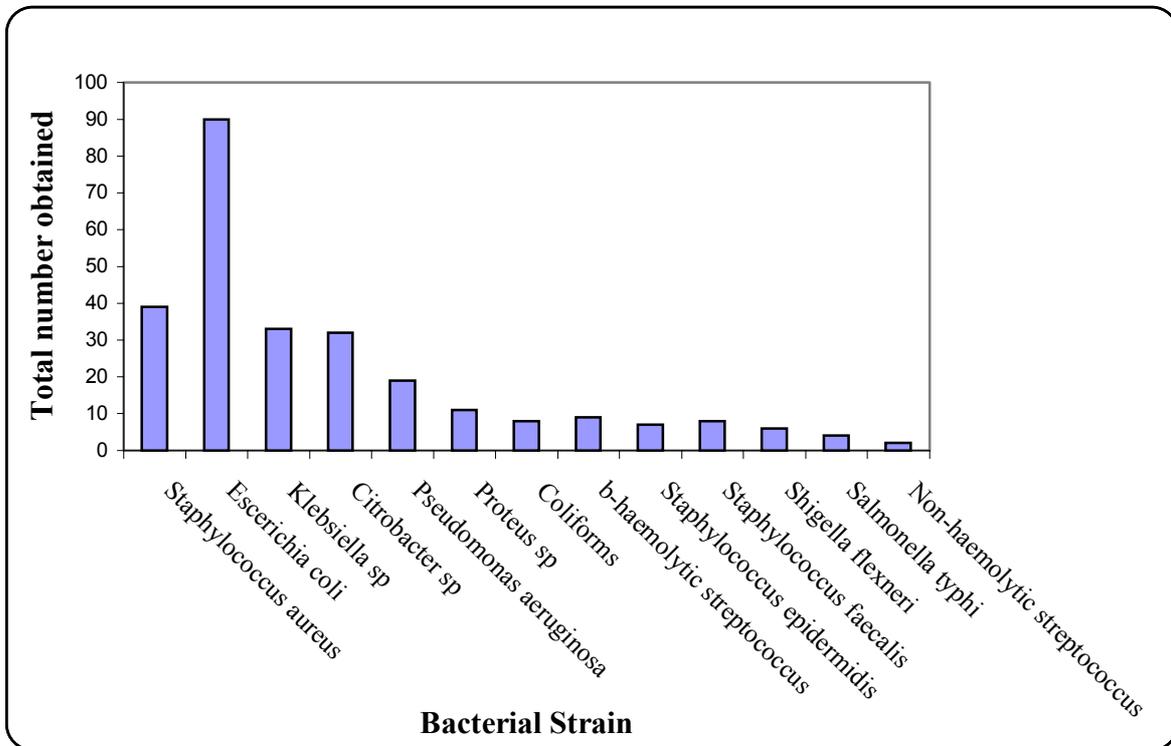**Figure 1: Types of culltures and freqency of cases**

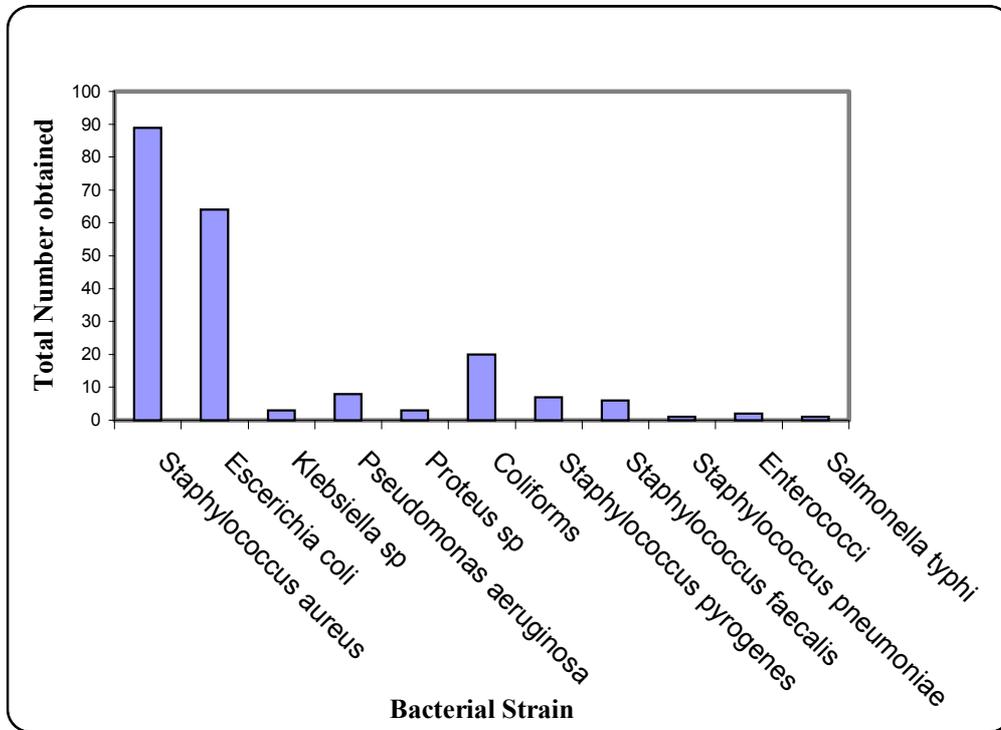**Figure 2a: microbes isolated from cultures at T/ Hospital**

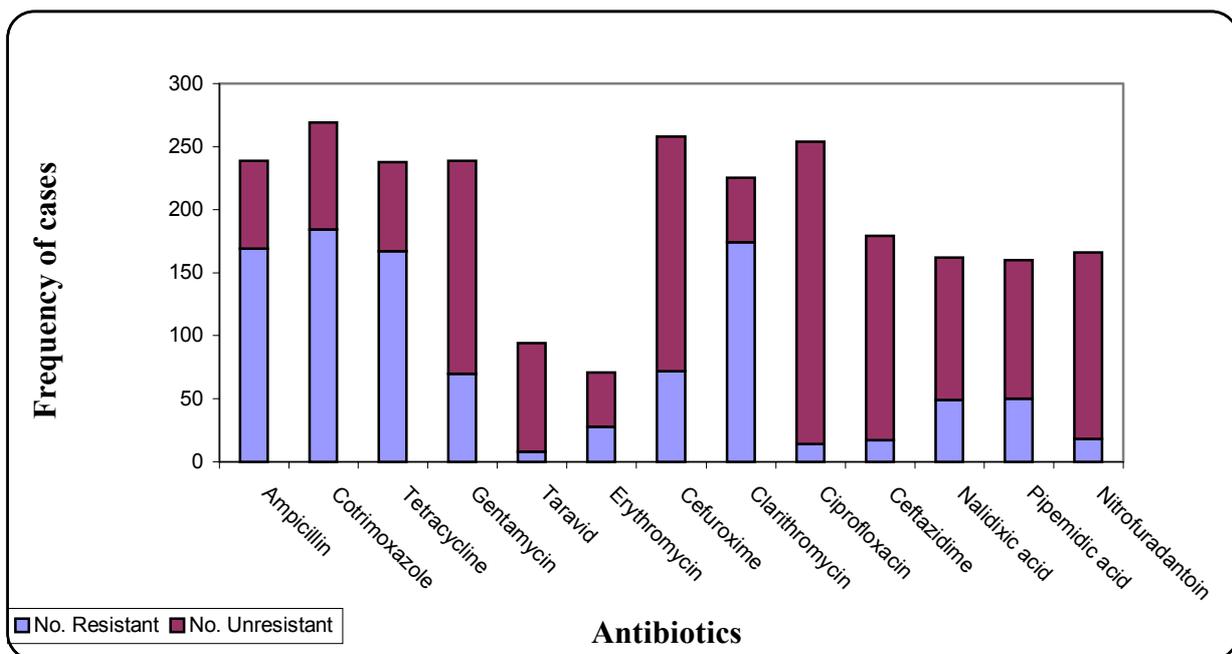**Figure 2b: microbes isolated from cultures at M /diagnositic Center**

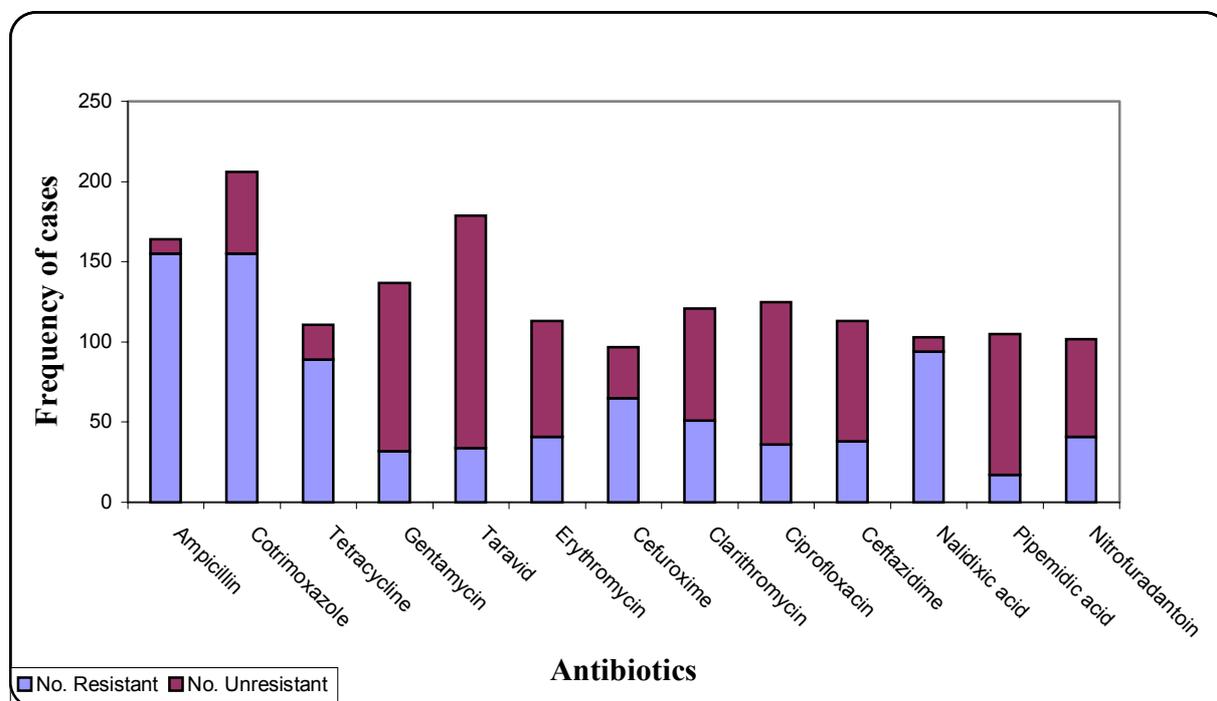**Figure 3a: Antibiotics Resistant pattern obtained at T/ Hospital**

Figure 3b: Antibiotics Resistant pattern obtained at M/ Diagnostic Center

Resistant by specific bacteria

Some microbes were resistant to specific antibiotics. *Staphylococcus aureus* was resistant to ampicillin (97%), cotrirmaxazole (89%), tetracycline and moderately resistant to gentamycin (36%) and erythromycin (36%). *Klebsiella spp* was resistant to ampicillin and moderately resistant to cotrimaxazole (33%), tetracycline (33%) and gentamycin. *Streptococcus pyogens* was resistant to ampicillin , cotrimoxazole, tetracycline and moderately resistant to gentamycin (40%). Salmonella typhi was moderately resistant to ampicillin, cotrimaxazole, tetracycline and gentamycin (data not shown).

Statistical analyses

The results from the two laboratories were compared using the student t-test. The t-value calculated was less than t critical at 5% level (tcal = 0.41 < tcri = 2.0). This revealed that the hypothesis No = N1 is useful, suggesting that the results from the two laboratories were similar, thus following the same trend.

DISCUSSION

The incidence of antibiotic resistance was confirmed in this study. Microbes were found to have high resistance to tetracycline, ampicillin and cotrimazaole, whereas streptomycin and erythromycin were moderately resisted. Gentamycin, a narrow spectrum antibiotic, was found to be effective against bacteria especially in treating urinary tract infection, pnuemonia and meningitids. Normally antibiotic should be effectively used within 2 years of general application. Unlike penicillin, some modern antibiotics have high endurance to microbial resistance. Vanomycin was used as a last line of defence for more than 30 years against multiple antibiotic resistant. Currently, resistant strains to Vanomycin have developed in the USA and even more in the intensive care unit of health facilities (9,10). Cephalosporin, having a low endurance, has faced rapid microbial resistance(11).

Limited and isolated cases of antibiotic resistance for specific microbes have been reported in some developing countries. In Vietnam, most isolates from patients with

diarrhoea were found resistant to majority of the commonly used antibiotics tested against them; 90% were resistant to 3 or more antibiotics (11). In India, *Pseudomonas aeruginosa* isolates form hospitalized patients were shown to be multi-drug resistant to 7 or more antibiotics tested, and even to amikacin(3). Previous antimicrobial studies in Africa were in isolated areas. *Bacillus anthrax* isolates from patients in South Africa and Zimbabwe were observed to have no respond to Cephalosporin in vitro(12). Antibiotic resistant isolates appears to be spreading fast in developing countries and even more alarming is the acquired resistant to multiple drugs and less frequently used drugs. More studies are necessary to confirm the incidence and resistant pattern of antibiotic strains in order to provide a baseline information for policy makers.

Numerous reasons can be assigned for the development of antibiotic resistance strains. In developing countries due to unaffordable laboratory tests and consultation fees, it is becoming popular to prescribe broad-spectrum antibiotics that are active against a wide range of different microbes. Such antibiotics exposes much more bacteria than is needed. Prescribing antibiotics that are more powerful than necessary could be harmful as for example bacteria is becoming insensitive to vanomycin, reserved antibiotic for serious infections. Too much antibiotic in animal feed to prevent intestinal infections could rapidly cause resistance to develop and passed on to humans as in *E. coli* (5). The rapid development of antibiotic resistance by *Shigella* and *Salmonella* may have develop in this way. Lack of adequate hospital control procedures may also result in the spread of infectious diseases caused by *Staphylococcus aureus*, *Salmonella spp.* and *Klebseilla spp*.

Comparison of the antibiotic resistance pattern results obtained from the two separate laboratories in the study area was necessary to find the relationship between them. Six antibiotics, of the same make and brand, tested in the two laboratories and compared using the student t-test, revealed that the two sets of results were similar statistically; ie they followed the same pattern. This suggests that the findings obtained from the different laboratories were not influenced significantly by environmental factors such as human. Many factors such as the type of equipment used, the method adopted, reagent applied, and the experimental duration could influence the results from different laboratories. This finding is important in formulating a protocol for mass monitoring of antibiotic resistance in the country and elsewhere. Mapping out the distribution of antibiotic resistant strains in a country requires a coordinated surveillance which must be backed with a mathematical model that detects similarities or otherwise

of results from different places. More studies are therefore required to understand and confirm the use of a statistical approach in an antibiotic surveillance program since in this study a small sample size and limited laboratories were involved.

A few numerical differences and contradictions in the levels and distribution of isolates resistance to antibiotics from the different laboratories were seen especially for some specific microbes. *Staphlococcus aureus* was 40% and 97% resistant to ampcillin, and 30% and 68% to cotrimaxazole, whereas *Salmonella typhi* was 100% and 50% resistant to tetracycline. The variation could be due to reasons such as differences in equipment used, reagent kit, judgement of staff among others. Further enquiry by questionnaire revealed the classes of patients that visited the laboratories were different. One laboratory was visited by high class patients due to its location and cost of diagnosis and the second by middle class people. The classes of patients was determined by income levels. This finding reaffirms the importance of incooperating a mathematical and/or statistical formular in a national monitoring programs to compare results from different laboratories.

Microbial contaminants such as *Staphlococcus epidermis* isolated in the blood cultures is a flora of the skin. The bacteria may probably infect the blood stream from endocarditis conditions and the enrichment media may enhance its growth. Secondly, the increasing use of intravenous catheters, interarterial lines and vascular prothese could increase its access to the blood stream. Furthermore, *S. epidermis* found in the urine is unusual but certain practices like oral sex, insertion of fingers into vagina and sex with same partners may cause it. The spread of *E. coli* in the vagina is again unusual since bacteria are cleansed after urinating. Probably the practice of cleaning the female private parts from back to front (from vagina to anus), introduces feacal bacteria into the vagina. In addition, splashes of water from closets during defecation may spread feacal pathogens to the vagina. Health education may be necessary to reduce pathogenic and contaminated non-pathogenic microbes to reduce health cost and disease burden.

CONCLUSION

This study shows the trend of microbial resistant strains to some commonly used antibiotics in parts of Accra metropolis, a Ghanaian community. It further reveals that differences in the results from different laboratories were statistically insignificant, indicating similarities in trend. The study provides an important baseline data for

formulating a protocol to monitor antibiotic resistance in the country.

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