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Screening of the Efficacy of Some Commonly Used Antibiotics in Ghana

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Abstract: The objective of this study was to screen some commonly used antibiotics in Ghana for their efficacy in treating diseases so as to select sensitive organisms that can be used to design an assay in assessing their biological activity. The disc susceptibility test was used to screen stock antibiotics such as ampicilline, chloramphenicol, kanamycin and penicillin based antibiotics from different manufacturers (both local and foreign) which were obtained from different pharmacy shops against some bacteria species such as *Salmonella typhi*, *Staphylococcus aureus* and six strains of *Escherichia coli*. It was observed that both stock and field antibiotics (Antibiotics obtained from pharmacy shops for study) zone of inhibition were similar and compared with literature values. J916 (an *E. coli* isolate) and *Salmonella typhi* were found to be less sensitive to the penicillin-based antibiotics similar to literature values for both stock and pharmacy shop samples. This study revealed that the antibiotics produced by local and foreign pharmaceutical companies appear to be effective. In as much as this study demonstrate that, local and foreign pharmaceutical industries appear to be producing quality drugs, further studies are needed to substantiate this claim observed by this study, which was on a small scale.

Key words: Ghana, disc susceptibility test, zone of inhibition, field antibiotic

INTRODUCTION

Since the discovery of antibiotics and knowledge of their potential in the field of medicine by curing certain bacteria infection, their production and synthesis have increased over the years especially in the USA (Antibacterials, 2006). In the period of 1956 through 1961 in the USA the sales value of antibiotics represented more than 50% of the sales value of all medicinal compounds. The same can be said of other countries but statistics are not available to prove their prevalence. It is not surprising that the same trend is observed in Ghana as there is influx of antibiotics into the Ghanaian market from pharmaceutical companies both foreign and local. As a result abuse of antibiotics has increased considerably due to the Over-The-Counter (OTC) services without prescription from a qualified physician.

With the open market policy of importation of drugs, the Ghanaian market is replete with antibiotics from manufacturers in both developed and developing countries. Antibiotics lose their potency with poor storage and depending upon condition of storage loss of biological activity of these antibiotics could be earlier than the expiry date that appears on the label. Therefore, it is extremely important to have a system to monitor the potency of antibiotics from different manufacturers that are found in pharmacy shops as well as chemical sellers shops with a view of comparing the quality

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of these products. In Ghana, most of the commonly used antibiotics are chloramphenicol (prescribed frequently to treat typhoid, meningitis and other related infections); ampicillin and penicillin based ones (treat wound infections).

In view of the sub-standards drugs produced by some pharmaceutical companies and indiscriminate use thereof, antibiotic resistant is becoming a public health problem in recent times for both hospital and community acquired infections (Garau *et al.*, 1999; Pederson *et al.*, 1999). Antimicrobial resistant Urinary Tract Infections (UTIs) and others have continued to increase at an alarming rate over the past 15 years (Astal *et al.*, 2002). There are many reasons for these alarming phenomena, including antibiotic use in animal feeds, inappropriate prescribing of antibiotics, poor infection control strategies (Astal *et al.*, 2002) and poor quality control methods employed by Pharmaceutical companies.

The defining property of an antibacterial agent is its ability to selectively interfere with bacterial growth and/or survival. Consequently, a considerable and crucial part of the preclinical evaluation of any novel antibacterial drug involves judging and characterizing its effects on bacteria *in vitro*. These critical stages in drug development are sometimes made to appear somewhat trivial, sandwiched as they are between the highly demanding antibacterial discovery process and the formidable task of demonstrating safety and efficacy *in vivo*. However, careful biological evaluation *in vitro* is key to quantifying and understanding the basis of the antibacterial activity (O'Neill and Chopra, 2004) providing preliminary indications and evaluations of therapeutic potential, assessing the likelihood for the development of bacterial resistance, guiding chemical refinement and assisting subsequent stages of the appraisal of any new antibacterial drug.

Whether in humans or animals, the antibiotic resistance of biofilms has a significant impact on health including increased morbidity and mortality (Livermore, 2003). The prolonged treatment of diseases and infections causes increased health costs and serious implications for both human and animal welfare. Currently, antibiotic selection is based on an antibiotic sensitivity test using the Kirby-Bauer disc diffusion method, developed in 1966 by Bauer and others (Bauer *et al.*, 1966). Other methods have since been developed but the disc diffusion technique was adopted by the National Committee for Clinical Laboratory Standards [NCCLS] in 1975 and is still used today as the basis for disc diffusion standards (Wheat, 2001).

In this study, the disc diffusion method was used to assess the biological activity of the antibiotics. This biological activity assessment of antimicrobial will be helpful for the Quality Control Units of the Ghana Standards Board and the Food and Drugs Board which are charged with this responsibility for screening antimicrobial. The study was therefore aimed at the search for sensitive organisms that can be used to assess the potency of antibiotics and design an assay system, using the sensitive organisms for the assessment of the potency of antibiotics from different manufactures that are found on the Ghanaian market. This method of antimicrobial sensitivity testing has been described as a reliable, easy and inexpensive method of evaluating antimicrobial efficacy (Gaudreau and Gilbert, 1997; Victor, 1981). In addition disc diffusion method is useful as a preliminary screening for susceptibility testing (Manoharan *et al.*, 2003), despite reservations against the disc diffusion method (Victor, 1981; Costerton *et al.*, 2003).

MATERIALS AND METHODS

Antibiotics Used and Preparations

The study was carried out January-March 1998, in Accra, Ghana. Ampicillin, chloramphenicol and kanamycin were obtained as stock antibiotics, which were kindly donated by Dr Yaa Difie Osei of the Biochemistry Department, University of Ghana. Penicillin based antibiotics such as cloxacillin capsule, amoxicillin capsule, ampicillin capsule and injection, crystalline and prochaine penicillin,

benzyl penicillin injection and chloramphenicol injection, were purchased from various pharmacy shops in Accra. Samples were checked for their batch numbers, manufacturer's origin and date of expiry. The antibiotics were dissolved in sterilized distilled water to make a stock concentration of $50 \mu\text{g}/20 \mu\text{L}^{-1}$. To increase the solubility, three to four drops of 25% NH_4OH and sterilized by filtering with 200 nm pore size membrane filter.

Preparation and Impregnation of Antimicrobial Discs

Discs of diameter 6.0 mm were punched from a sheet of Whatman Number 3 filter paper (UK) by a perforator and arranged in petri dishes allowing a distance of 2- 4 mm between each of them. The discs were sterilized in an oven at 160.0°C for 15 min. To check for sterility 4 discs were randomly selected and placed on Mueller Hinton Agar (MHA) in a petri dish and incubated at 37.0°C in the incubator overnight. Signs of growth around the disc meant it was not properly sterilized and were discarded. However, if no growth was seen around them, then the discs were properly sterilized. After the discs were allowed to cool (attaining room temperature), the penicillin antibiotic discs were separately impregnated with $10 \mu\text{g}$ each, while kanamycin and chloramphenicol discs were impregnated $30 \mu\text{g}$ each. The impregnated discs were arranged in separate petri dishes and dried by placing them in an incubator at temperature of 37°C for 2-3 h.

Culturing of Organism and Sensitivity Test

Six strains of *Escherichia coli* (*E. coli*) were obtained from the Bacteriology Unit of the Noguchi Memorial Institute for Medical Research, Accra, Ghana. These strains were given code such as 06775 ST, J916, 955LT, J1060, ATCC 25922, 101695 ST. Two other clinical specimens *Salmonella typhi* (*S. typhi*) and *Staphylococcus aureus* (*S. aureus*) were obtained from the Biochemistry Department, Legon, Ghana. These strains of bacteria were stock-cultured in peptone water. Mueller Hinton Agar was prepared for the sensitivity test. For good standardization to attain reproducible results, the following measures were taken during the sensitivity test:

- The discs diameters were uniform, 6.0 mm in diameter
- Volume of agar poured was about 15 mL for 90.0 mm diameter Petri dishes
- Equal volume of peptone-water (10 mL) was measured into the culturing tube
- Bacteria inoculum of 0.5 MacFarland was prepared by picking a colony of the organism into the peptone-water using sterile streaking loop
- The prepared Mueller Hinton agar plates were dried before they were inoculated
- The volume of culture used to inoculate the agar in the plates was $500.0 \mu\text{L}$
- Sub culturing of pure culture was done at least once a week to avoid any contamination
- For long storage the microorganisms were transferred unto agar slopes and then incubated overnight of 37°C . Glycerol was added unto the surface of agar slope with microbial growth

In performing the sensitivity test $20.0 \mu\text{L}$ of the inoculum was poured gently onto a dried prepared Mueller Hinton Agar in an agar plate using a sterile micropipette. The plate was gently rotated for uniform distribution on the inoculums on the medium. After the inoculation the impregnated disc was picked by a sterile forceps and placed gently unto the inoculated agar. Susceptibilities to various antibiotics were determined by modified Kirby-Bauer disk diffusion methods according to the clinical Laboratory Standards Institute as described elsewhere (Ceri *et al.*, 1999).

Zone of Inhibition Test for Stock and Field Antibiotics

Solution of ampicillin, chloramphenicol and kanamycin were prepared from the stock solution as described earlier to obtain the following concentration per disc 10.0 , 30.0 and $30.0 \mu\text{g}$, respectively.

After inoculation for about 15 min, each of the impregnated discs was placed gently unto the inoculated agar. The distance between each impregnated disc on agar plate was about 5cm and approximately 15 mm from edge of plate as described by Clutterbuck *et al.* (2007). The agar plates were incubated overnight at a temperature of 37°C. Reasonable zones of inhibition were measured to establish which microorganisms were sensitive to the stock antibiotics. The procedure was repeated once to obtain reliable and valid results. The Penicillin based antibiotics that were used were ampicillin, amoxicillin, cloxacillin and penicillin. They were prepared with the same method to have concentration of 10 µg each and the susceptibility testing performed with the same bacteria species.

Statistical Analysis

Data were entered into Excel programme and statistical analysis done using the one-way ANOVA. Values were considered significant when p value was less than 0.05.

RESULTS AND DISCUSSION

Table 1 gives a summary of zone of inhibition against 10 µg AM, 30 µg KA and 30 µg CH. *S. typhi* and *S. aureus* did not respond to 30 µg CH concentration. Also strains of *E. coli* were relatively more sensitive to 30 µg CH whereas, J1060LT was resistant to 10 µg AM. *S. typhi* and *S. aureus* did not show any zone of inhibition for cloxacillin (data not shown).

Evaluating Potency of Field Antibiotics

In order to evaluate the potency of the field antibiotics against Gram negative bacteria, *S. typhi* and J916 were used as representative clinical microorganism for the screening of the field antibiotics. The sensitivity test and zone of inhibition to ampicillin and amoxicillin antibiotics from pharmacy shops are shown in Table 3. Both show similar show zone of inhibition to the selected microorganisms except zone of inhibition by ampicillin (CD 0201 by a local company), which was higher compared to amoxicillin (1000D01 also from local company), in relation to J916. Again for J916, zone of inhibition for ampicillin (D 594001, from a foreign company) was less than that of amoxicillin (AX 33A, from a local company), (Table 3). The zone of inhibition for the different forms of penicillin preparations of the field antibiotic are summarized in (Table 4). Except for benzyl procaine penicillin

Table 1: Mean (±SD) zones of inhibition around all the Stock Antibiotics against microorganisms provided

Microorganism	Concentration of stock antibiotics and zone of inhibition (mm±SD)		
	AM (10 µg)	KA (30 µg)	CH (30 µg)
<i>Salmonella typhi</i>	23.4±0.7 ^S	21.2±0.5 ^S	0 ^R
<i>Staphylococcus aureus</i>	18.3±0.8 ^R	20.6±0.4 ^S	0 ^R
309775 ST	15.2±0.9 ^S	26.3±0.8 ^S	24.1±0.6 ^S
J916	17.7±0.8 ^S	23.2±0.7 ^S	25.2±0.7 ^S
955 LT	19.6±0.7 ^S	23.9±0.6 ^S	28.2±0.6 ^S
J1060 LT	0 ^R	23.4±0.9 ^S	25.2±0.8 ^S
ATCC 25922	16.5±0.6 ^S	ND	28.6±0.7 ^S
101695 ST	0 ^R	ND	ND

AM: Ampicillin; CH: Chloramphenicol; KA: Kanamycin; 0 implies no inhibition; SD: Standard deviation; ND: Not done; S: Sensitive; R: Resistant. NB: Each value was the mean of 4 readings and the expiry dates of the stock antibiotics were between May-June 2001

Table 2: Range of zone of sensitivity of some antibiotics against gram negative and positive bacteria species

Type of bacteria	Penicillin (10 µg)	CH (30 µg)	KA (30 µg)	AM (10 µg)	AMX (10 µg)
Gram positive	≥ 29 mm	≥ 18 mm	≥ 29 mm	≥ 29 mm	≥ 29 mm
Gram negative	≥ 20 mm	≥ 18 mm	≥ 14 mm	≥ 14 mm	≥ 14 mm
Type of bacteria	References				
Gram positive	http://pathmicro.med.sc.edu/major/antibiot.htm				
Gram negative	http://pathmicro.med.sc.edu/major/antibiot.htm				

CH, KA, AM and AMX have their usual meaning

Table 3: Mean (\pm SD) mm zones of inhibition around different states of Penicillin Injection Preparations antibiotics from different manufactures against selected microorganisms

Ampicillin (10 μ g)		
Microorganism	B No. and manufacturer	ZI (mm)
<i>Salmonella typhi</i> J916	CD 0201, (LPC-B, Accra, Ghana; Exp. date: Jan 2000)	19.7 \pm 0.5 ^S
	D 594001, (FPC, Germany; Exp. date: Apr 1998)	20.3 \pm 0.4 ^S
	AM 71A, (LPC-A, Accra, Ghana; Exp. Date: Jan 2000)	22.8 \pm 0.4 ^S
	CD 0201, (LPC-B, Accra, Ghana; Exp. Date: Jan 2000)	23.2 \pm 0.8 ^S
	D 594001, (FPC, Germany; Exp. Date: Apr 1998)	16.8 \pm 0.4 ^S
	AM 71A, (LPC-A, Accra, Ghana; Exp. Date: Jan 2000)	24.2 \pm 0.4 ^S
Amoxicillin (10 μ g)		
Microorganism	B No. and manufacturer	ZI (mm)
<i>Salmonella typhi</i> J916	1000D01, (LPC-C, Accra, Ghana; Exp. Date: Feb 2001)	20.5 \pm 0.5 ^S
	AX33A, (LPC-A, Accra, Ghana; Exp. Date: Nov 2000)	19.0 \pm 0.5 ^S
	1395, (FPC, India; Exp. Date: Oct 2000)	22.5 \pm 0.5 ^S
	MD 7355, (FPC, Italy; Exp. Date: Apr 2000)	21.3 \pm 0.7 ^S
	1000D01, (LPC-C, Accra, Ghana; Exp. Date: Feb 2001)	14.0 \pm 0.5 ^S
	AX33A, (LPC-A, Accra, Ghana; Exp. Date: Nov 2000)	23.3 \pm 0.4 ^S
	1395, (FPC, India; Exp. Date: Oct 2000)	24.5 \pm 0.5 ^S
	MD 7355, (FPC, Italy; Exp. Date: Apr 2001)	24.3 \pm 0.4 ^S

BNo.: Batch number; ZI: Zone of Inhibition; S: sensitive; R: Resistant; NM: Not measured; FPC: Foreign Pharmaceutical Company. Exp. Date: Expiry date. NB: Each value was the mean of 4 readings

Table 4: Mean (\pm SD) mm zones of inhibition around different states of Penicillin Injection Preparations antibiotics from different manufactures against selected microorganisms

Crystalline penicillin injection preparation (10 μ g)		
Microorganism	B No. and manufacturer	ZI (mm)
<i>Salmonella typhi</i> J916	1503-02, (FPC, Germany; Exp. Date: Mar 2000)	19.5 \pm 0.5 ^S
	1503-02, (FPC, Germany; Exp. Date: Mar 2000)	18.0 \pm 0.4 ^R
Benzyl penicillin injection preparation (10 μ g)		
Microorganism	B No. and manufacturer	ZI (mm)
<i>Salmonella typhi</i> J916	5-501, (FPC, India; Exp. Date: Jun 1998)	20.8 \pm 0.8 ^S
	1098, (LPC-D, Accra, Ghana; Exp. Date: Apr 2000)	19.0 \pm 0.5 ^R
	5-501, (FPC, India; Exp. Date: Jun 1998)	NM
	1098, (LPC-D, Accra, Ghana; Exp. Date: Apr 2000)	17.5 \pm 0.5 ^R
Procaine penicillin injection preparation (10 μ g)		
Microorganism	B No. and manufacturer	ZI (mm)
<i>Salmonella typhi</i> J916	1537-03, (FPC, Germany; Exp. Date: Sep 2000)	20.3 \pm 0.8 ^S
	1537-03, (FPC, Germany; Exp. Date: Sep 2000)	14.3 \pm 0.4 ^R

BNo.: Batch number; ZI: Zone of Inhibition; S: Sensitive; R: Resistant; NM: Not measured; FPC: Foreign Pharmaceutical Company. Exp. Date: Expiry date. NB: Each value was the mean of 4 readings

injection (5-501 from a foreign company) whose zone of inhibition could not be measured against J916, the others showed some zone of inhibition but were below literature values (Clutterbuck *et al.*, 2007; Table 2). This may probably be due to the gram negative nature of these microorganisms. The other observation made was that cloxacillin did not show any zone of inhibition whatsoever (data not shown). However, 5-501 and 1537-03 also from a foreign company were sensitive to *S. typhi*.

Clinical laboratories have used antibiotic diagnostic devices as guides to selection of therapy nearly as long as such drugs have been commercially produced. And in this study, the disc diffusion testing, which was used to screen for the biological activity of the field antibiotics, gave results which are reproducible, thus indicating the quality of the field antibiotics. And that the significance of this study is such that the biological activity of the antibiotics can be carried out to assess the potency of field antibiotics to complement the chemical activity. In addition, the results obtained are of a high standard which has a reasonable degree of accuracy and it is the common laboratory test for antibiotic susceptibility (Livermore, 2003; Bauer *et al.*, 1966; Victor, 1981), compared with agar dilution.

However, one major disadvantage of the agar dilution compared with disc diffusions is the fact that test plates cannot be sub cultured easily in order to determine the bactericidal activity of the antimicrobial agent. In view of the above reasons the disc diffusion has been the most widely used procedure. Furthermore, the suitability of Mueller Hinton agar for antimicrobial sensitivity testing, stemmed from the fact that it is found not to contain inhibitory substances to antimicrobial compounds and its pH for routine sensitivity work is 7.2-7.4.

In the sensitivity test of the stock antibiotics, the ampicillin stock preparation did show a relative wide spectrum of biological activity. Most of the microorganisms were sensitive to it with the exception of J1060 LT and 101695 ST. Ampicillin is generally active against gram-negative and gram-positive bacteria (Clutterbuck *et al.*, 2007). The resistance of J1060 LT and 101695 ST to ampicillin could be that these strains of *E. coli* may have produced beta-lactamase or acid that hydrolyses the ampicillin thereby losing its activity.

It is surprising to note that *S. aureus* was resistant to chloramphenicol. This is because $1 \mu\text{g mL}^{-1}$ chloramphenicol inhibits most gram positive bacteria and many gram-negative bacteria are inhibited by concentrations of $0.2\text{-}5 \mu\text{g mL}^{-1}$ (Katzung, 1992). In this study, however, when compared to the other two antibiotic stocks (ampicillin and kanamycin) it is found that the strains of *E. coli* were more sensitive to chloramphenicol (Table 1). The fact that J1060 was sensitive to both chloramphenicol and kanamycin but not ampicillin emphasizes the point mentioned earlier that J1060 may have produced beta-lactamase or acid that hydrolyzed the ampicillin structure.

In the sensitivity test of some of the antibiotics from the pharmacy shops, the zone of inhibition for J916 (a strain of *E. coli*) and *S. typhi* were comparable and similar to the stock as well, which were comparable to literature. It has also been shown that, there are instances when such organisms become resistant to lower concentrations, which we observe in preliminary findings of this study. Other possibility is that the growth of the bacteria might be in a biofilm state, which has been found to be recalcitrant to antibiotic treatment (Ceri *et al.*, 1999), as a result the choice and concentration of antibiotic are often unsuccessful and must be increased.

The fact that the zone of inhibition of the stock and the field antibiotic were similar could imply that the potency of the antibiotic by the pharmaceuticals was of good quality. However, the difference in the zone of inhibition of amoxicillin made by one local Pharmaceutical company in Accra on J916 was 14.0 ± 0.5 mm as against the stock of 17.1 ± 0.5 mm, were statistically significant ($p < 0.05$). Since, studies has shown that a $10 \mu\text{g}$ disc concentration of antibiotic giving a zone of inhibition of more than 14mm is sensitive, we can conclude that the stock is more sensitive compared to the one by the pharmaceutical company, which is also effective. Furthermore the zone of inhibition by the stock of 23.4 ± 0.7 mm by ampicillin (Table 1) and that of amoxicillin by the same local pharmaceutical company in Accra was 20.5 ± 0.5 mm (Table 3) to *S. typhi*, suggesting that the antibiotics will be effective in the clearance of microorganisms during an infection.

One other clear observation made was the lack of sensitivity of *S. typhi* and J916 to cloxacillin. This was not unusual due to the fact that isoxazolyl penicillins such as cloxacillin have no activity against such gram-negative bacteria. However, these isoxazolyl penicillins are used in combination with either ampicillin or one of the cephalosporins for the treatment of urinary tract infections caused by gram-negative bacilli (Astal *et al.*, 2002). The function of the isoxazolyl penicillin is to protect the action by binding bacteria beta-lactamases which destroy these drugs (Bennet and Kuccers, 1979). It is possible to obtain high value of zones of inhibition with diameters of 30-35 mm, which are arbitrarily indicative of high susceptibility and zones of up to 15 mm and are related to organisms resistant to the drug concentration employed (Jorgensen and Ferraro, 2000). Exceptions to this generation are those organisms whose zones of inhibition are approximately 20 mm when tested with penicillin and which must be checked for penicillinase production.

Within experimental error, zone of inhibition by the penicillin antibiotics (namely benzyl penicillin injection, crystalline penicillin injection and procaine penicillin) were similar. However, benzyl penicillin injection made in India (Table 4) did not inhibit the growth of J916 and not indicative of the potency of benzyl penicillin injection being lost or of low quality, as it has been shown to have inhibited growth of *S. typhi*. There could be possible implication of penicillinase production as its zone of inhibition on J916 was below 20 mm. Date of manufacture and expiratory date of almost all the field antibiotic were the same and had no effect on the potency of the antibiotic.

Taken together, the results in this study is significant in that the inhibition of growth by both stock and field antibiotics enabled the selection of microorganisms that can be used to design an assay system to examine the biological activity of antibiotics.

CONCLUSION

The strains of *E. coli* were sensitive to ampicillin stock with the exception of J1060 LT. The zone of inhibition of the field antibiotic and that of the stock did not vary much, when compared to that in literature, affirming that this method of screening antibiotics from the pharmacy shops is reliable, thus the biological activity assay can be designed to assess the potency of the antibiotics. In as much, this study demonstrate that, local and foreign pharmaceutical industries appear to be producing quality drugs, further studies are needed to substantiate this claim observed by this study, which was on a small scale.

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