Pharmaco bacteriological investigation of erythromycin, cephalaxin, cephradine, amoxycillin and ciprofloxacin against different strains of *klebsiella pneumoniae*, *escherichia coli*, *pseudomonas aeruginosa*, *staphylococcus aureus* and *salmonella* species

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Abstract

Different groups of antibiotics (quinolones, macrolides, penicillin and cephalosporins) were chosen and used in this study. These antibiotics included ciprofloxacin, erythromycin, amoxycillin, cephalaxin and cephradine respectively. Twenty six bacterial species were collected from urine, milk, rectal swabs, liver, wounds, intestine and also from isolated culture which were supplied by the department of microbiology, faculty of veterinary medicine, university of Khartoum, Sudan. Identification was done by using identification kits namely Quick GN “Nissui” and also by using biochemical tests as confirmatory tests. The bacterial species were found to be: *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and six strains of *Salmonella* species. Sensitivity tests were performed for all these organisms against various antibiotics with different concentrations using standard disk diffusion method. Ciprofloxacin was found to be the most effective drug against all the organisms tested even at a very low concentration (0.781 µg /ml), all the Gram-negative bacteria were found to be resistant to erythromycin. *Pseudomonas aeruginosa* strains were found to be highly sensitive to ciprofloxacin and resistant to the most of the other groups of antibiotics. Quinolon group (Ciprofloxacin, ofloxacin and pefloxacin) was found to be the most effective group against *Salmonella typhi*

Keywords: Erythromycin, Cephalexin, Cephradine, Amoxycillin, Ciprofloxacin, *E. coli*, *Klebsiella*, *Salmonella* spp.

Introduction

Antibiotics are extensively used to combat infectious diseases of bacterial origin.

Quinolones carboxylic acid derivatives are synthetic anti-microbial agents that are becoming more important in veterinary medicine. Older members of this class of synthetic antimicrobial agents, particularly Nalidixic acid, have been available for the treatment of urinary tract infections for many years. These drugs are of relatively minor significance because of their limited therapeutic utility and the rapid development of bacterial resistance.

Ciprofloxacin is one of the most active fluorinated quinolones. It is also active against *Mycobacterium leprae* as well as *M. tuberculosis*.

Pefloxacin Mesylate is a fluoroquinolone antibacterial agent with actions and uses similar to those of ciprofloxacin.

The cephalosporins are bactericidal and similarly to the penicillins, they act by inhibiting synthesis of the bacterial cell wall.

In general, cephalosporins are active *in-vitro* against many bacterial species and they are usually bactericidal in action. The antibacterial activity of cephalosporins, like penicillins, I-oxa-β-lactams, carbacephems, and cephamycins,

Amoxycillin is bactericidal for both Gram-positive and Gram-negative organisms. As it is destroyed by β-lactamase, the drug is ineffective in many *Staphylococcal* infections.

The macrolides are a large group of antibiotics mainly derived from *Streptomyces* spp. Their properties are very similar and in general they have low toxicity and the same spectrum of antimicrobial activity with cross-resistance between individual members of the group. The macrolide are bacteriostatic or bactericidal depending on the concentration and the type of micro-organism. Their antimicrobial spectrum is similar to that of benzylpenicillin but they are also active against such organisms like *Legionella pneumophila*, *Mycoplasma pneumoniae*, and some rickettsias and chlamydias.

Erythromycin and other macrolides blockage the transpeptidation or translocation reactions, inhibition of protein synthesis, and hence inhibition of cell growth.

The current research aimed to investigate the antibacterial activity and to evaluate the susceptibility of different bacterial species against different antibiotics.

Materials and Methods

Bacterial strains were obtained from animal infections and the isolated strains were supplied by the department of microbiology, Faculty of Veterinary Medicine, University of Khartoum, Sudan. For identification of Gram-negative bacteria quick GN (Nissui) and some other additional biochemical tests were used. The primary and secondary biochemical tests were used to identify the Gram-positive bacteria according to Barrow and Feltham.

Antimicrobial susceptibility test was done on Mueller–Hinton agar (Oxoid, England) using disk diffusion technique according to Kirby–Bauer method. The antimicrobial agents tested were: Erythromycin, cephalexin, cephradine, Amoxycillin and ciprofloxacin in different concentrations (50, 25, 12.5, 6.25, 3.125, 1.563 and...
0.781 µg). The discs of different antibiotics were placed on the plates that had been previously inoculated and flooded by an overnight culture of nutrient broth after it was diluted.

Results and Discussion

Twenty-one bacteria species and strains were classified according to the Kits and biochemical properties (E. coli, Klebsiella pneumoniae, Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus). The antibiotic sensitivity results were shown in tables (1, 2, 3, 4 and 5).

Ciprofloxacin is bactericidal at or near the minimum inhibitory concentration for the most common Gram-negative pathogens. It is also verified that ciprofloxacin inhibited the most species of Enterobacteriaceae at concentration 1 mg/l. All the E. coli strains were found to be highly sensitive to ciprofloxacin at different concentrations ranging between 0.781 µg - 50 µg but resistant to erythromycin. Enterobacteriaceae were found to be highly sensitive to ciprofloxacin but not to erythromycin and this is due to production of erythromycin esterases. Fluoroquinolones inhibited many types of Gram-negative bacteria including Enterobacteriaceae at concentration of 0.1 – 5 µg/ml.

Ciprofloxacin was found to be the most effective drug against E. coli and these results were also simulating another research by Charlton et al. Dollery et al mentioned that the appearance of ampicillin resistance among many of Enterobacteriaceae made amoxycillin a less attractive choice for the management of common infective conditions.

In this study, three strains of E. coli (60%) were found to be sensitive to amoxycillin at concentrations ranging between 6.25 µg – 50 µg, but two strains (40%) remained resistant. Four strains of E. coli (80%) were found to be resistant to amoxycillin at concentrations ranged between 0.781 µg – 3.125 µg and only one strain was found to be moderately sensitive at concentration of 3.125 µg and resistant at concentrations of 0.781 and 1.563 µg. From the above results it would be seen that amoxycillin was not effective against E. coli. This rise in the resistance reduced the value of amoxycillin to be used in the treatment of urinary tract infection caused by E. coli.

Two strains of E. coli (40%) were found to be sensitive to cephalaxin at concentration ranging between 25 µg – 50 µg and resistant at concentrations ranging between 0.781 µg – 12.5 µg. Two strains (40%) were found to be sensitive to cephalaxin at concentrations ranging between 0.781 µg – 3.125 µg.

Many scientists are of the same opinion as they verified that first generation cephalosporins are usually active in-vitro against Gram-positive cocci and they have limited activity against Gram-negative bacteria, but some strains of E. coli may be inhibited in-vitro by the drug. Two strains of E. coli were also found to be sensitive to cephradine at concentrations ranging between 6.25 µg – 50 µg and two other strains were found to be moderately sensitive at the same concentrations. Only one strain of E. coli was found to be sensitive at concentrations ranging between 25 µg – 50 µg. In general, first generation cephalosporins are active in-vitro against Gram-positive cocci and some strains of E. coli may be inhibited in-vitro by cephradine at concentrations 0.8-12.5 µg/ml. It was observed that cephalaxin and cephradine were not active in-vitro at concentrations ranging between 0.781 – 3.125 µg/ml although some strains were found to be resistant even at high concentration (12.5 µg/ml). The major mechanism of bacteria resistance to cephalosporines is the production of β-lactamases. However, absence or presence of β-lactamases does not entirely indicate susceptibility or resistance to cephalosporins.

Previous studies have also shown very high resistance against cephalosporins and penicillins. Most E. coli strains were multiple drug resistance and 5 isolates were extensively drug resistant. Multiple drug resistance was defined as resistance to three or more than three different antibiotic classes tested.

Klebsiella Strains: All the strains of Klebsiella were found to be highly sensitive to ciprofloxacin and resistant to erythromycin and amoxycillin at concentrations ranging between 0.781 µg – 50 µg/ml. The most strains of Enterobacteriaceae are highly susceptible to ciprofloxacin. This result is in agreement with that ciprofloxacin had excellent activity (100% susceptibility) against K. pneumoniae however, in another research 31.7% of K. pneumoniae isolates were found to be resistant to ciprofloxacin.

Enterobacteriaceae are not susceptible to erythromycin, and this fact was supporting the result in this study. Klebsiella spp. are generally resistant to a wide range of antibiotics, and naturally resistant to ampicillin.

It was observed that Klebsiella was not sensitive to amoxicillin. Three strains of Klebsiella (60%) were found to be moderately sensitive, to sensitive to cephalaxin at concentrations ranging between 3.125 µg–50 µg/ml and two strains were found to be moderately sensitive, to sensitive at concentrations ranging between 6.25 µg/ml – 50 µg/ml. Four strains of Klebsiella were found to be moderately sensitive to sensitive to cephradine at concentrations ranging between 6.25 µg - µg 50 µg/ml and only one strain was found to be resistant to cephradine. The most strains of Klebsiella were inhibited by cephradine or cephalaxin at concentration ranging between 6.25 µg/ml–50 µg/ml. However, susceptible strains of E.coli and Klebsiella pneumoniae are generally inhibited by cephradine or cephalaxin at concentrations of 0.8 – 12.5 µg/ml. A significant difference between cephradine and cephalaxin, regarding to their activity in-vitro was observed. It was observed that Klebsiella strain 6 is sensitive to cephalaxin and resistant to cephradine.

Salmonella Strains: All the strains of Salmonellae were found to be sensitive to ciprofloxacin and resistant to erythromycin at concentrations ranging between 0.781 µg/ml – 50 µg/ml. This result is in agreement with another study. Also ciprofloxacin is the drug of choice for salmonellae infections. In this study ciprofloxacin resistance was not detected. It was concluded that
ciprofloxacin was the most active *in-vitro* drug against salmonella strains.

Erythromycin and other macrolides have no useful activity against enteric Gram-negative bacilli. It has been asserted that Enterobacteriaceae are not susceptible to erythromycin, and these findings support the results of this study.

Amoxicillin was found to be very active *in-vitro* against *Salmonella dublin* at concentrations ranging between 1.563-50 µg/ml.

Two strains of *Salmonella* (50%) were found to be sensitive to amoxicillin at concentrations ranging between 6.25µg/ml–50 µg/ml and resistant at concentrations ranging between 0.781-3.125 µg/ml. One strain of *Salmonella* (25%) was found to be sensitive to amoxicillin at concentrations ranging between 3.125µg/ml – 50 µg/ml and moderately sensitive at concentrations ranging between 0.781µg/ml-1.563 µg/ml. One strain of *Salmonella* (25%) was found to be resistant to amoxicillin at concentrations ranging between 0.781µg/ml – 50 µg/ml. From the above results it was observed that 60% of the strains of salmonella were resistant to amoxicillin at concentrations ranging between 0.781µg/ml– 3.125 µg/ml.

Therefore, there is an increasing percentage of *Salmonella spp*. that are not sensitive to amoxicillin.

*Salmonella dublin* was found to be sensitive to cephalexin at concentrations ranging between 6.25 µg/ml–50 µg/ml, moderately sensitive at concentration 3.125 µg/ml, and resistant at concentrations 0.781 µg/ml and 1.563 µg/ml. Cephradine was found to be active against *Salmonella dublin* at concentrations ranging between 6.25 µg/ml – 50 µg/ml and resistant at concentrations ranging between 0.781µg/ml-3.125 µg/ml. This result showed no big difference between the activity of cephalexin and cephradine.

Three strains of *Salmonella* (75%) were found to be sensitive to cephalexin and cephradine at concentrations ranging between 6.25-50 µg/ml, although cephradine is active against many strains of Enterobacteriaceae causing urinary tract infection, including *Salmonella spp.*

**Pseudomonas Strains:** All the strains of *Pseudomonas* were found to be sensitive to ciprofloxacin at concentrations ranging between 0.781 µg/ml – 50 µg/ ml. Four strains of *Pseudomonas* (80%) were found to be resistant to erythromycin, amoxicillin, cephalexin and cephradine. Only one strain was found to be moderately sensitive to cephalexin and cephradine at concentrations ranging between 3.125 µg/ml – 50 µg/ml and was found to be moderately sensitive to amoxicillin at concentrations 25 µg/ml and 50 µg/ml, but also resistant to erythromycin at concentrations ranging between 0.781 µg/ml – 25 µg/ml. Our results supported by other research of El-karsh et al. It was found that 100% of *Pseudomonas aeruginosa* strains were sensitive to ciprofloxacin and this result is in agreement with Indudharan et al and not disagree with Kozlova et al.

Also Mascellino et al found that 50% of *Pseudomonas aeruginosa* strains remained susceptible to ciprofloxacin and Hanberger et al found that 37% of *Pseudomonas aeruginosa* strains were resistant to ciprofloxacin. No resistant strain of *Pseudomonas aeruginosa* to ciprofloxacin.

The most *Pseudomonas aeruginosa* strains were resistant to ampicillin and this result is in agreement of the findings in this study.

Cephalexin was inactive against *Pseudomonas aeruginosa* and the resistance was mainly due to it is partially the result of ß-lactamase production by the microorganisms. *Pseudomonas* had maximum resistance against ciprofloxacin, levofloxacin, norfloxacin, ofloxacine, and moxifloxacin, while it was highly susceptible to tazobactam/piperacillin.

**Staphylococcus Strain:** All the strains of *Staphylococcus aureus* were found to be highly sensitive to ciprofloxacin at concentrations ranging between 0.781 µg/m – 50 µg/m. This result is in agreement with that of Walfsen et al. The *in-vitro* activity of ciprofloxacin against *Staphylococcus aureus* is consistent regardless of whether organisms are methicillin-resistant or not. However, there is 89% of methicillin-resistant *Staphylococcus aureus* were resistant to ciprofloxacin. The resistance rates to erythromycin was 80% in methicillin-resistant *Staphylococci* and about 30% in methicillin-sensitive *Staphylococci*. It was found 100% of *Staphylococcus aureus* were sensitive to ciprofloxacin, although Baiocchi et al observed significant increased resistance of *Staphylococcus aureus* to ciprofloxacin.

Two strains of *Staphylococcus aureus* (40%) were found to be sensitive to erythromycin at concentrations ranging between 1.563 µg/m–50 µg/ml and two strains (40%) were found to be resistant.

Most strains of *Staphylococcus aureus* were sensitive to erythromycin. In this study only one strain was found to be sensitive to erythromycin at concentrations ranging between 6.25 µg/m –50 µg/ml and moderately sensitive at concentrations 1.563 µg/ml and at 3.125 µg/ml. From the above results it was observed that 60% of *Staphylococcus aureus* were found to be sensitive to erythromycin at concentrations ranging between 6.25µg/m–50 µg/m. These results are in agreement of Chang et al.

Amoxicillin is used in the treatment of infections caused by susceptible Gram-positive bacteria including *Staphylococcus*. In this study three strains (60%) of *Staphylococcus aureus* were found to be sensitive to amoxicillin at concentrations ranging between 1.563 µg/m – 50 µg/m and only one strain was found to be sensitive at concentrations ranging between 6.25 µg/m – 50 µg/m. Only one strain was found to be resistant to amoxicillin at concentrations ranging between 0.781 µg/m - 50 µg/m.

*Staphylococcus aureus* strains were susceptible to cephalexin *in-vitro* and this findings is in agreement with the result of this study. Also all strains of *Staphylococcus aureus* were found to be susceptible (sensitive and moderately sensitive) to cephalexin and cephradine at concentrations ranging between 3.125 µg/m – 50 µg/ml.

In general first generation cephalosporins are active, *in-vitro*, against Gram-positive cocci and cephalexin is the...
most effective treatment of infections caused by *Staphylococcus aureus*.11,22,43

98% of *Staphylococcus aureus* were eradicated by using cephalexin as a treatment of skin infections and it was very effective in the treatment of infections caused by *Staphylococcus aureus*44,46 and these findings supporting the results of this study, in which it was found that all the strains of *Staphylococcus aureus* were susceptible to cephalexin and cephradine. It is also found that the susceptibilities of *S. aureus* strains to tetracycline, rifampin, ciprofloxacin, gentamicin and TMP-SMX were 56%, 59%, 56%, 56% and 99%, respectively.47

Table 1

| Antibiotics | Means of zone inhibitions of different antibiotics against different strains of *E.Coli* |
|-------------|--------------------------------------------------------------------------------------|
|             | E.Coli1     | E.Coli2     | E.Coli3     | E.Coli4     | E.Coli5     |
| Ciprofloxacin | 29.571 A   | 29.571 A   | 27.000 A   | 29.571 A   | 30.4286 A   |
| Cephalexin   | 29.571 D   | 29.571 C   | 14.286 B   | 15.571 C   | 17.0000 B   |
| Cephradine   | 9.286 C    | 5.714 C    | 13.714 B   | 18.429 BC  | 16.0000 B   |
| Amoxycillin  | 19.143 B   | 17.0000 B  | 0.0000 C   | 22.671 B   | 0.0000 C    |
| Erythromycin | 3.714 D    | 0.0000 D   | 0.0000 C   | 7.857 D    | 0.0000 C    |

Means followed by the same letter are not significantly different at P=0.01

Table 2

| Antibiotics | Means of zone inhibitions of different antibiotics against different strains of bacteria |
|-------------|--------------------------------------------------------------------------------------|
|             | Klep6       | Klep7       | Klep8       | Klep9       | Klep10      |
| Ciprofloxacin | 24.1429 A   | 25.1430 A   | 25.4290 A   | 26.2860 A   | 25.7140 A   |
| Cephalexin   | 14.7143 B   | 17.5710 B   | 16.5710 B   | 16.2860 B   | 14.5710 B   |
| Cephradine   | 12.0000 C   | 14.5710 C   | 16.000 B    | 13.4290 C   | 14.1430 B   |
| Amoxycillin  | 3.8571 D    | 12.5710 C   | 8.2860 C    | 0.0000 C    | 0.0000 C    |
| Erythromycin | 0.0000 E    | 0.0000 D    | 0.0000 D    | 0.0000 C    | 0.0000 C    |

Means followed by the same letter are not significantly different at P=0.01

Table 3

| Antibiotics | Means of zone inhibitions of different antibiotics against different strains of *Salmonella* |
|-------------|------------------------------------------------------------------------------------------------|
|             | *Salmonella Dublin* | *Salmonella 20* | *Salmonella 30* | *Salmonella 40* | *Salmonella 50* |
| Ciprofloxacin | 29.4290 A   | 30.0000 A   | 31.0000 A   | 29.8571 A   | 29.8570 A    |
| Cephalexin   | 14.7140 B   | 18.8571 C   | 15.7140 B   | 18.2857 C   | 3.2860 D     |
| Cephradine   | 13.5710 B   | 19.0000 C   | 15.2860 B   | 17.8571 C   | 9.2860 C     |
| Amoxycillin  | 28.0000 A   | 27.2867 B   | 31.1430 A   | 27.2857 B   | 12.7140 B    |
| Erythromycin | 0.0000 C    | 0.0000 C    | 0.0000 C    | 0.0000 C    | 0.0000 C     |

Means followed by the same letter are not significantly different at P=0.01

Table 4

| Antibiotics | Means of zone inhibitions of different antibiotics against different strains of *Pseudomonas* |
|-------------|------------------------------------------------------------------------------------------------|
|             | *Pseudomonas 1* | *Pseudomonas 2* | *Pseudomonas 3* | *Pseudomonas 4* | *Pseudomonas 5* |
| Ciprofloxacin | 26.4286 A   | 26.8571 A   | 26.8571 A   | 28.1430 A   | 26.8571 A    |
| Cephalexin   | 0.0000 B    | 0.0000 B    | 0.0000 B    | 13.2860 C   | 0.0000 B     |
| Cephradine   | 0.0000 B    | 0.0000 B    | 0.0000 B    | 15.2860 C   | 0.0000 B     |
| Amoxycillin  | 0.0000 B    | 0.0000 B    | 0.0000 B    | 17.1430 C   | 0.0000 B     |
| Erythromycin | 0.0000 B    | 0.0000 B    | 0.0000 B    | 9.2860 C    | 0.0000 B     |

Means followed by the same letter are not significantly different at P=0.01

Table 5

| Antibiotics | Means of zone inhibitions of different antibiotics against different strains of *Staphylococcus* |
|-------------|------------------------------------------------------------------------------------------------|
|             | *Staph A* | *Staph B* | *Staph C* | *Staph D* | *Staph E* |
| Ciprofloxacin | 24.4286 B | 23.5710 B | 29.8571 A | 25.0000 B | 22.5714 A |
| Cephalexin   | 19.0000 C | 17.5710 C | 18.8571 C | 15.5714 E | 18.1429 B |
| Cephradine   | 19.2857 C | 18.8570 C | 17.5714 C | 17.7143 D | 18.0000 B |
| Amoxycillin  | 32.8571 A | 28.2860 A | 24.2857 B | 31.5714 A | 16.4286 C |
| Erythromycin | 0.0000 D  | 9.7140 B  | 22.8571 A | 21.7143 A | 22.5714 A |

Means followed by the same letter are not significantly different at P=0.01
Conclusion
Ciprofloxacin is highly effective against Gram-positive and Gram-negative bacteria in-vitro at concentration less than 1µg/ml, and also it is the drug of choice for treatment of Typhoid fever. *Pseudomonas aeruginosa* is susceptible to ciprofloxacin and resistant to cephalosporins, erythromycin and amoxycillin. While Enterobacteria are not susceptible to erythromycin. Also in this study it was found that *Klebsiella strains* were resistant to amoxycillin.

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