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Original article

Susceptibility pattern of enteric bacteria isolated during raining season in some areas of Ado-Ekiti to macrolide antibiotics

Oluwafemi Adeyemi Ajenifuja *, Oluwakemi Esther Oni

Department of Science Technology, School of Science and Computer Studies, Federal Polytechnic, Ado-Ekiti, Nigeria.

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ABSTRACT

Background: Macrolides are considered one of the oldest classes of antibiotics which have been regarded among the best-tolerated antibiotic for almost several years. They are characterized by their large lactone ring structures and by their growth-inhibiting (bacteriostatic) effects on bacteria. **Aim:** The potency of macrolide antibiotics were determined against enteric bacteria (*E. coli, Shigella* spp. and *Salmonella* spp.) isolated during the raining season between June to September in the year 2018 from locations in Ado Ekiti metropolis. The consequences of the presence of these bacteria can be fatal hence the need to investigate their susceptibility to macrolide antibiotics. **Methods:** The bacteria were isolated from well water, soil and drainage samples obtained at Erifun, Omisanjana, Fiyinfoluwa, Ajilosun areas using serial dilution method. **Results:** It was observed that the bacteria were susceptible to 250 µg/mL and 500 µg/mL concentration of azithromycin, erythromycin, and clarithromycin though with varying degrees of susceptibility. Azithromycin showed the highest potency. **Conclusion:** The present study indicated samples of the well water, soil and drainage at Erifun, Omisanjana, Ajilosun and Fiyinfoluwa areas of Ado-Ekiti were severely contaminated with *E. coli*, *Salmonella* spp., and *Shigella* spp. this is due to the lack of adequate sanitary measures. However, unhygienic behaviour like indiscriminate disposal of waste and open defecation should be discouraged.

Introduction

Macrolides are class of antibiotics characterized by their large lactone ring structures and by their growth-inhibiting (bacteriostatic) effects on bacteria. The macrolides were first discovered in the 1950s, when scientists isolated erythromycin from the soil bacterium *Streptomyces erythraeus*. In the 1970s and 1980s synthetic derivatives of erythromycin, including clarithromycin and azithromycin, were developed [1]. Macrolides are usually administered orally, but they can be given parenterally. They are used in treating pneumonias caused either by *Mycoplasma* species or by *Legionella pneumophila* (the organism that causes Legionnaire disease); they are also used in treating pharyngeal carriers of *Corynebacterium diphtheriae*, the bacillus responsible for diphtheria [2].

Antibiotic macrolides are used to treat infections caused by Gram-positive bacteria such as *Streptococcus pneumoniae* and limited Gram-negative bacteria which are *Bordetella pertussis*, *Haemophilus influenzae*, and some respiratory tract and soft-tissue infections. The antimicrobial spectrum of macrolides is slightly wider than that of penicillin, and, therefore, macrolides are a common substitute for patients with a penicillin allergy [3]. Macrolides have been reported to exhibit activity against many Gram-positive

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* *Corresponding author:* Oluwafemi Adeyemi Ajenifuja
E-mail address: joseyajenifuja@yahoo.com

bacteria excluding enterococci and methicillin-resistant *Staphylococcus aureus* [4], and also have variable activity against respiratory Gram-negative pathogens such as *Mycobacterium avium* infections, gonorrhea [1].

Enteric bacteria naturally live in the intestines of animals and humans. However, some types of bacteria reside in intestinal tracts of animals that can cause disease and harsh reactions when humans become infected with them. They can cause a mild infection, such as a simple case of food poisoning, or they can cause severe community-wide infections and lead to plagues. Examples of enteric bacteria include *Salmonella* spp., *E. coli*, *Campylobacter jejuni* and *Shigella dysenteriae* [5].

The primary means of bacterial resistance to macrolides occurs by post-transcriptional methylation of the 23S bacterial ribosomal RNA [6]. This acquired resistance can be either plasmid-mediated or chromosomal, that is, through mutation, and results in cross-resistance to macrolides, lincosamides, and streptogramins. Azithromycin has been used to treat strep throat (Group A streptococcal infection caused by *Streptococcus pyogenes*) in penicillin-sensitive patients, however, macrolide-resistant strains of Group A streptococci are not uncommon [6].

Macrolides have a common structure formed by a large lactone ring [7], and this may partially explain its intrinsic activity against Gram-negative bacteria. Macrolide antibiotics inhibit Gram-negative bacteria by binding reversibly to the P site on the 50S subunit of the bacterial ribosome [7]. Macrolides are actively concentrated within leukocytes, and thus are transported into the site of infection.

Enteric bacteria find their way into water systems by the activities of man and animal in form of fecal pollution, they are abundant in drainage systems as a result of the release of sewage and other waste materials, and they are also found in abundance in the soil. Therefore, the aim of this study is to determine the potency of macrolide antibiotics against enteric bacteria isolated from well-water, drainage, and soil during the raining season.

Material and Methods

The study area and collection of samples

The study was carried out in four locations in Ado-Ekiti metropolis **(Figure 1)**. The locations include Erifun, Omisanjana, Fiyinfoluwa and Ajilosun. Samples were obtained from different well water, soils and drainages. These samples were obtained during the raining season between June and September.

Preparation of medium

The method described by **Ajibade et al.** [8] was adopted. Culturing of samples was done on McConkey agar. The agar was prepared according to the manufacturer's instructions. 3.9 g of McConkey agar was dissolved in 100 mL of distilled water, and then autoclaved in an electronic autoclave for 15 minutes at a temperature of 121^{o}C. The molten agar was allowed to cool to 45^{o}C and aseptically poured into sterile Petri dishes and allowed to solidify before use.

Culturing of the samples

The method described by **Ajibade et al.** [8] was adopted. Serial dilutions of the samples were made in test tubes to obtain a dilution factor of 10^5. Half mL of the dilution factor was streaked evenly onto the surface of a properly labeled solidified overdried McConkey agar plates. The plates were inverted and incubated at 37°C for 18 hours. Discrete colonies were picked, sub-cultured and stored in the refrigerator on a nutrient agar slant.

Reactivation and identification of bacterial isolates

The method described by **Ajibade et al.** [8] was adopted Colonies were picked with a flamed inoculated loop and cultured in the test tube of McConkey broth, incubated in an incubator at 37°C for 18 hours. Subsequently, a loop full of the suspension was streaked on an overdried McConkey agar and incubated at 37°C for 24 hours.

The pure bacterial isolates were identified based on their morphological and biochemical tests such as pigmentation, shape, elevation, consistency, margin, Gram staining, catalase test, fermentation of sugars, indole production and sensitivity tests [9]. In order to determine the identity of bacteria isolates, results were compared with standard references of Bergey's Manual of Determinative Bacteriology as described by **Buchanan and Gibbons** [10].

Concentration of macrolide antibiotics

The method described by **Khan et al.** [11] was adopted. Four different macrolide antibiotics (azithromycin, clarithromycin and erythromycin) were ground into powder form in different containers. Two different concentrations of the macrolide antibiotic were made for each of the antibiotics i.e. 250 and 500 µg/mL, and dissolved in 1 mL of distilled water.

Impregnation of the paper disk

Disk diffusion method described by **Khan et al.** [11] was adopted. Paper discs were prepared from Whatman filter No. 1 filter paper (5 mm) and then sterilize in the hot air oven for 60 ^{o}C for 1 hr. The disks

were incorporated into the different concentrations of the prepared macrolide antibiotics and were allowed to stand for 24 hours at room temperature.

Antibiotic susceptibility test

The guideline described by Clinical Laboratory Standards Institute (CLSI) [12] was adopted. Reactivated bacterial suspensions were spread evenly on the prepared nutrient agar using a sterile wire loop. Macrolide antibiotic discs were placed on the plates in three different locations. The plates were properly labeled and incubated at 37ºC for 24 hours. Zones of inhibition/susceptibility patterns were measured and recorded in millimeters.

Figure 1. Map of Ado-Ekiti showing the study areas

Satistical analysis

Analysis of variance was computed using Statistical Package for the Social Sciences (SPSS) 15 software for each attribute and the Duncan multiple range test was used to separate the means where significant difference existed.

Ethical approval

All authors hereby declare that all research methodologies have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Results

The results of this work are shown in the tables below. **Table 1** shows the prevalence of the different bacterial isolates from each sample for each months (June – September). The three bacterial were isolated in June in the soil sample; while two (*Salmonella* spp. and *Shigella* spp.) were isolated from drainage and well water samples respectively. The samples collected in July, August and September had two bacterial isolates each. There was a significant difference between the samples.

The result of the susceptibility pattern of bacterial (*Salmonella* spp., *E. coli*, and *Shigella* spp.) isolated from Erifun in June – September is showed in **table (2)**. During June, the isolates from well-water were susceptible to the three macrolides with a diameter of zone of inhibition ranging between 9.00 – 28.00 mm, 8.00 – 36.00 mm, and 5.00 – 37.00 mm respectively. The isolates from the soil sample were susceptible to the three macrolides with a diameter of zone of inhibition ranging between 6.00 – 32.00 mm, 7.00 – 36.00 mm, and 8.00 – 36.00 mm respectively; isolates from drainage were also susceptible to the three macrolides with a diameter of zones of inhibition between 8.00 – 36.00 mm, 8.00 – 33.00 mm, and 6.00 – 32.00 mm respectively at 250 – 500 µg/mL concentrations. There was a significant difference ($p < 0.05$) between the samples.

The result of the susceptibility pattern of bacterial (*Salmonella* spp., *E. coli*, and *Shigella* spp.) isolated from Omisanjana in June – September is showed in **table (3)**. During June, the isolates from well-water were susceptible to the three macrolides with a diameter of zone of inhibition ranging between 7.00 – 29.00 mm, 7.00 – 30.00, and 9.00 – 37.00 mm respectively. The isolates from soil sample were susceptible to the three macrolides with a diameter of zone of inhibition ranging between 7.00 – 28.00 mm, 6.00 – 32.00 mm, and 9.00 – 38.00 mm respectively; isolates from drainage were also susceptible to the three macrolides with the diameter of zones of inhibition between 8.00 – 36.00 mm, 8.00 – 33.00 mm, and 10.00 – 36.00 mm respectively at 250 – 500 µg/mL concentrations. There was a significant difference ($p < 0.05$) between the samples.

The result of the susceptibility pattern of bacterial (*Salmonella* spp., *E. coli*, and *Shigella* spp.) isolated from Ajilosun in June – September is showed

in **table (4)**. During June, the isolates from well-water were susceptible to the three macrolides with a diameter of zone of inhibition ranging between 9.00 – 36.00 mm, 10.00 – 43.00 mm, and 7.00 – 29.00 mm respectively. The isolates from the soil sample were susceptible to the three macrolides with a diameter of zone of inhibition ranging between 9.00 – 30.00 mm, 10.00 – 35.00 mm and 9.00 – 39.00 mm respectively; isolates from drainage were also susceptible to the three macrolides with a diameter of zones of inhibition between 10.00 – 33.00 mm, 8.00 – 33.00 mm, and 7.00 – 33.00 mm respectively at 250 – 500 µg/mL concentrations. There was a significant difference ($p< 0.05$) between the samples.

The result of the susceptibility pattern of bacterial (*Salmonella* spp., *E. coli*, and *Shigella* spp.) isolated from Fiyinfoluwa in June – September is showed in **table (5)**. During June, the isolates from well-water were susceptible to the three macrolides with a diameter of zone of inhibition ranging between 7.00 – 43.00 mm, 10.00 – 37.00 mm, and 6.00 – 35.00 mm respectively. The isolates from soil sample were susceptible to the three macrolides with a diameter of zone of inhibition ranging between 7.00 – 32.00 mm, 6.00 – 36.00 mm, and 8.00 – 37.00 mm respectively; isolates from drainage were also susceptible to the three macrolides with the diameter of zones of inhibition between 6.00 – 40.00 mm, 5.00 – 30.00 mm, and 7.00 – 36.00 mm respectively at 250 – 500 µg/mL concentrations. There was a significant difference ($p< 0.05$) between the samples.

Table 1. Prevalent bacteria from samples.

Months	**Samples**		
	Organisms isolated		
	Soil	**Drainage**	**Well water**
June	*E, Sal, Sh*	*E, Sal*	*Sal, Sh*
July	*Sal, Shi*	*Sal, Sh*	*Sal, Sh*
August	*E, Sal*	*E, Sal*	*E, Sal*
September	*Sal, Sh*	*Sal, Sh*	*E, Sal*

E – *Escherichia coli,* Sal – *Salmonella* spp., Sh – *Shigella* spp.

Table 2. Susceptibility patterns of bacterial isolates in Erifun**.**

Macrolides/ Sources	**Diameter of zone of inhibition (mm)**							
	Concentration of antibiotics (µg/mL)							
	June		July		August		September	
	250	500	250	500	250	500	250	500
***Salmonella* spp.**								
Well water								
Azithromycin	28.00±0.01^{a}	24.00±0.02^{b}	29.00±0.01^{c}	33.00±0.00^{d}	24.00±0.01^{b}	26.00±0.01^{e}	21.00±0.02^{f}	23.00±0.01^{g}
Clarithromycin	16.00±0.02^{a}	18.00±0.03^{b}	15.00±0.00^{c}	16.00±0.03^{a}	12.00±0.01^{d}	14.00±0.02^{e}	8.00±0.04^{f}	12.00±0.00^{d}
Erythromycin	11.00±0.01^{a}	12.00±0.01^{b}	12.00±0.01^{b}	13.00±0.01^{c}	10.00±0.00^{d}	11.00±0.01^{a}	9.00±0.03^{e}	12.00±0.02^{b}
Soil								
Azithromycin	25.00±0.01^{a}	29.00±0.01^{b}	32.00±0.01^{c}	36.00±0.01^{d}	27.00±0.02^{e}	28.00±0.03^{f}	24.00±0.01^{g}	27.00±0.02^{e}
Clarithromycin	9.00±0.02^{a}	12.00±0.03^{b}	6.00±0.02^{c}	8.00±0.01^{d}	11.00±0.00^{e}	13.00±0.02^{f}	10.00±0.03^{g}	11.00±0.00^{e}
Erythromycin	8.00±0.01^{a}	10.00±0.01^{b}	7.00±0.01^{c}	9.00±0.01^{d}	11.00±0.01^{e}	12.00±0.01^{f}	12.00±0.01^{f}	13.00±0.01^{g}
Drainage								
Azithromycin	32.00±0.01^{a}	35.00±0.01^{b}	34.00±0.00^{c}	36.00±0.02^{d}	26.00±0.04^{e}	30.00±0.01^{f}	32.00±0.00^{a}	33.00±0.01^{g}
Clarithromycin	14.00±0.01^{a}	16.00±0.02^{b}	12.00±0.02^{c}	14.00±0.02^{a}	8.00±0.01^{d}	10.00±0.01^{e}	11.00±0.01^{f}	12.00±0.00^{c}

Erythromycin	8.00±0.01^{a}	9.00±0.03^{b}	9.00±0.02^{b}	11.00±0.01^{c}	11.00±0.00^{c}	13.00±0.02^{d}	8.00±0.03^{a}	11.00±0.01^{c}
E. coli								
Well water								
Azithromycin	32.00±0.02^{a}	34.00±0.00^{b}	26.00±0.01^{c}	27.00±0.01^{d}	31.00±0.01^{e}	34.00±0.01^{b}	26.00±0.01^{c}	36.00±0.01^{f}
Clarithromycin	15.00±0.01^{a}	16.00±0.01^{b}	12.00±0.01^{c}	15.00±0.01^{a}	11.00±0.01^{d}	13.00±0.02^{e}	12.00±0.02^{c}	16.00±0.00^{b}
Erythromycin	15.00±0.01^{a}	13.00±0.01^{b}	14.00±0.01^{c}	15.00±0.01^{a}	9.00±0.01^{d}	12.00±0.01^{e}	8.00±0.01^{f}	9.00±0.01^{d}
Soil								
Azithromycin	34.00±0.01^{a}	36.00±0.02^{b}	27.00±0.01^{c}	31.00±0.01^{d}	36.00±0.01^{b}	37.00±0.00^{e}	32.00±0.00^{f}	34.00±0.00^{a}
Clarithromycin	7.00±0.01^{a}	10.00±0.00^{b}	6.00±0.02^{c}	8.00±0.03^{d}	11.00±0.01^{e}	12.00±0.01^{f}	12.00±0.01^{f}	14.00±0.01^{g}
Erythromycin	10.00±0.00^{a}	12.00±0.01^{b}	8.00±0.02^{c}	9.00±0.03^{d}	11.00±0.01^{e}	12.00±0.02^{b}	9.00±0.00^{d}	10.00±0.02^{a}
Drainage								
Azithromycin	24.00±0.01^{a}	32.00±0.01^{b}	32.00±0.01^{b}	33.00±0.01^{c}	28.00±0.01^{d}	29.00±0.00^{e}	30.00±0.01^{f}	32.00±0.01^{b}
Clarithromycin	16.00±0.01^{a}	16.00±0.01^{a}	13.00±0.01^{b}	14.00±0.01^{c}	12.00±0.02^{d}	13.00±0.01^{b}	11.00±0.03^{e}	14.00±0.01^{c}
Erythromycin	10.00±0.02^{a}	12.00±0.01^{b}	8.00±0.04^{c}	9.00±0.00^{d}	10.00±0.02^{a}	12.00±0.01^{b}	11.00±0.00^{e}	12.00±0.01^{b}
***Shigella* spp.**								
Well water								
Azithromycin	36.00±0.02^{a}	37.00±0.00^{b}	31.00±0.02^{c}	34.00±0.01^{d}	30.00±0.01^{e}	31.00±0.02^{c}	29.00±0.01^{f}	31.00±0.01^{c}
Clarithromycin	7.00±0.04^{a}	10.00±0.01^{b}	5.00±0.04^{c}	9.00±0.01^{d}	11.00±0.00^{e}	12.00±0.02^{f}	13.00±0.00^{g}	16.00±0.01^{h}
Erythromycin	11.00±0.02^{a}	12.00±0.00^{b}	11.00±0.03^{a}	11.00±0.02^{a}	9.00±0.01^{c}	10.00±0.01^{d}	11.00±0.01^{a}	12.00±0.01^{b}
Soil								
Azithromycin	28.00±0.01^{a}	29.00±0.01^{b}	22.00±0.02^{c}	26.00±0.01^{d}	28.00±0.02^{a}	29.00±0.03^{b}	33.00±0.01^{e}	36.00±0.02^{f}
Clarithromycin	11.00±0.00^{a}	13.00±0.01^{b}	9.00±0.04^{c}	12.00±0.02^{d}	8.00±0.01^{e}	11.00±0.01^{a}	11.00±0.01^{a}	12.00±0.02^{d}
Erythromycin	9.00±0.02^{a}	12.00±0.01^{b}	12.00±0.00^{b}	13.00±0.01^{c}	12.00±0.02^{b}	13.00±0.01^{c}	10.00±0.01^{d}	11.00±0.01^{e}
Drainage								
Azithromycin	19.00±0.02^{a}	22.00±0.00^{b}	25.00±0.01^{c}	30.00±0.01^{d}	31.00±0.01^{e}	32.00±0.01^{f}	28.00±0.01^{g}	29.00±0.01^{h}
Clarithromycin	11.00±0.00^{a}	12.00±0.02^{b}	8.00±0.00^{c}	10.00±0.04^{d}	8.00±0.01^{c}	9.00±0.02^{e}	13.00±0.03^{f}	14.00±0.02^{g}
Erythromycin	12.00±0.01^{a}	13.00±0.01^{b}	6.00±0.01^{c}	9.00±0.03^{d}	9.00±0.02^{d}	10.00±0.02^{e}	7.00±0.03^{f}	8.00±0.01^{g}

*average of quadruplet, *Values with the same superscripts in the same row are not significantly different ($p < 0.05$).*

Table 3. Susceptibility patterns of bacterial isolates in Omisanjana.

Macrolides/ Sources	Diameter of zone of inhibition (mm) Concentration of antibiotics (µg/mL)							
	June		July		August		September	
	250	500	250	500	250	500	250	500
***Salmonella* spp.**								
Well water								
Azithromycin	26.00±0.04^{a}	27.00±0.01^{b}	21.00±0.02^{c}	23.00±0.01^{d}	28.00±0.01^{e}	29.00±0.01^{f}	23.00±0.01^{d}	25.00±0.00^{g}
Clarithromycin	9.00±0.00^{a}	12.00±0.00^{b}	8.00±0.02^{c}	12.00±0.01^{b}	15.00±0.02^{d}	16.00±0.02^{e}	12.00±0.00^{b}	14.00±0.01^{f}
Erythromycin	9.00±0.00^{a}	10.00±0.01^{b}	6.00±0.01^{c}	8.00±0.01^{d}	7.00±0.01^{e}	9.00±0.00^{a}	13.00±0.01^{f}	15.00±0.03^{g}
Soil								
Azithromycin	19.00±0.01^{a}	22.00±0.00^{b}	24.00±0.04^{c}	28.00±0.00^{d}	28.00±0.01^{d}	29.00±0.01^{e}	26.00±0.02^{f}	27.00±0.03^{g}
Clarithromycin	10.00±0.01^{a}	11.00±0.01^{b}	13.00±0.02^{c}	15.00±0.01^{d}	10.00±0.00^{a}	11.00±0.02^{b}	8.00±0.00^{e}	9.00±0.03^{f}
Erythromycin	7.00±0.02^{a}	9.00±0.01^{b}	9.00±0.00^{b}	9.00±0.02^{b}	8.00±0.00^{c}	11.00±0.04^{d}	9.00±0.01^{b}	12.00±0.02^{e}
Drainage								
Azithromycin	31.00±0.03^{a}	35.00±0.01^{b}	32.00±0.00^{c}	33.00±0.01^{d}	34.00±0.00^{e}	36.00±0.02^{f}	32.00±0.03^{c}	35.00±0.02^{b}
Clarithromycin	9.00±0.01^{a}	10.00±0.01^{b}	13.00±0.00^{c}	15.00±0.01^{d}	14.00±0.01^{e}	17.00±0.00^{f}	18.00±0.02^{g}	19.00±0.03^{h}
Erythromycin	8.00±0.02^{a}	10.00±0.01^{b}	12.00±0.02^{c}	14.00±0.02^{d}	11.00±0.01^{e}	12.00±0.01^{c}	15.00±0.00^{f}	16.00±0.03^{g}
E. coli								
Well water								
Azithromycin	23.00±0.02^{a}	26.00±0.01^{b}	23.00±0.00^{a}	25.00±0.02^{c}	28.00±0.02^{d}	30.00±0.02^{e}	23.00±0.01^{a}	25.00±0.04^{c}
Clarithromycin	7.00±0.01^{a}	12.00±0.02^{b}	9.00±0.00^{c}	10.00±0.01^{d}	13.00±0.02^{e}	17.00±0.02^{f}	11.00±0.00^{g}	15.00±0.02^{h}
Erythromycin	9.00±0.00^{a}	11.00±0.01^{b}	10.00±0.02^{c}	18.00±0.02^{d}	12.00±0.02^{e}	15.00±0.02^{f}	10.00±0.02^{c}	13.00±0.02^{g}
Soil								
Azithromycin	23.00±0.01^{a}	27.00±0.00^{b}	22.00±0.02^{c}	25.00±0.00^{d}	22.00±0.02^{c}	27.00±0.01^{b}	28.00±0.01^{e}	32.00±0.02^{f}
Clarithromycin	11.00±0.02^{a}	15.00±0.01^{b}	9.00±0.02^{c}	12.00±0.01^{d}	11.00±0.01^{a}	14.00±0.02^{e}	8.00±0.01^{f}	10.00±0.02^{g}
Erythromycin	7.00±0.01^{a}	10.00±0.02^{b}	6.00±0.01^{c}	9.00±0.00^{d}	10.00±0.01^{b}	13.00±0.00^{e}	8.00±0.02^{f}	10.00±0.02^{b}
Drainage								
Azithromycin	28.00±0.00^{a}	33.00±0.02^{b}	27.00±0.02^{c}	31.00±0.01^{d}	31.00±0.02^{d}	34.00±0.01^{e}	28.00±0.02^{a}	29.00±0.00^{f}
Clarithromycin	8.00±0.00^{a}	12.00±0.01^{b}	11.00±0.01^{c}	13.00±0.00^{d}	15.00±0.03^{e}	17.00±0.01^{f}	12.00±0.01^{b}	15.00±0.01^{e}
Erythromycin	10.00±0.01^{a}	12.00±0.02^{b}	10.00±0.01^{b}	13.00±0.02^{c}	9.00±0.01^{d}	11.00±0.01^{e}	12.00±0.00^{b}	14.00±0.01^{f}
***Shigella* spp.**								
Well water								
Azithromycin	23.00±0.01^{a}	37.00±0.01^{b}	20.00±0.02^{c}	23.00±0.02^{a}	23.00±0.00^{a}	25.00±0.01^{d}	23.00±0.03^{a}	26.00±0.00^{e}
Clarithromycin	10.00±0.02^{a}	12.00±0.01^{b}	9.00±0.01^{c}	12.00±0.02^{b}	13.00±0.03^{d}	16.00±0.01^{e}	12.00±0.01^{b}	15.00±0.01^{f}
Erythromycin	16.00±0.00^{a}	18.00±0.01^{b}	9.00±0.02^{c}	12.00±0.02^{d}	14.00±0.00^{e}	17.00±0.02^{f}	13.00±0.01^{g}	15.00±0.00^{h}
Soil								
Azithromycin	29.00±0.00^{a}	32.00±0.02^{b}	34.00±0.01^{c}	38.00±0.03^{d}	28.00±0.01^{e}	23.00±0.01^{f}	26.00±0.02^{g}	32.00±0.01^{b}
Clarithromycin	11.00±0.01^{a}	13.00±0.02^{b}	13.00±0.01^{b}	15.00±0.03^{c}	10.00±0.02^{d}	13.00±0.02^{b}	11.00±0.01^{a}	14.00±0.01^{e}
Erythromycin	9.00±0.03^{a}	11.00±0.02^{b}	9.00±0.02^{a}	15.00±0.01^{c}	11.00±0.02^{b}	14.00±0.01^{d}	9.00±0.02^{a}	12.00±0.02^{e}
Drainage								
Azithromycin	30.00±0.01^{a}	32.00±0.02^{b}	28.00±0.00^{c}	33.00±0.03^{d}	32.00±0.01^{b}	36.00±0.02^{e}	31.00±0.01^{f}	32.00±0.00^{b}
Clarithromycin	10.00±0.01^{a}	10.00±0.02^{a}	13.00±0.01^{b}	15.00±0.01^{c}	14.00±0.03^{d}	17.00±0.00^{e}	18.00±0.01^{f}	19.00±0.02^{g}
Erythromycin	10.00±0.02^{a}	12.00±0.02^{b}	12.00±0.01^{b}	14.00±0.02^{c}	11.00±0.01^{d}	15.00±0.00^{e}	15.00±0.02^{e}	17.00±0.01^{f}

*average of quadruplet, *Values with the same superscripts in the same row are not significantly different ($p < 0.05$).*

Table 4. Susceptibility patterns of bacterial isolates in Ajilosun.

Macrolides/ Sources	Diameter of zone of inhibition (mm)							
	Concentration of antibiotics (µg/mL)							
	June		July		August		September	
	250	500	250	500	250	500	250	500
***Salmonella* spp.**								
Well water								
Azithromycin	25.00±0.02^{a}	27.00±0.01^{b}	19.00±0.02^{c}	23.00±0.02^{d}	25.00±0.02^{a}	28.00±0.02^{e}	33.00±0.02^{f}	36.00±0.02^{g}
Clarithromycin	9.00±0.01^{a}	11.00±0.02^{b}	9.00±0.02^{a}	12.00±0.02^{c}	13.00±0.00^{d}	14.00±0.01^{e}	12.00±0.03^{c}	15.00±0.02^{f}
Erythromycin	12.00±0.02^{a}	14.00±0.01^{b}	10.00±0.02^{c}	12.00±0.02^{a}	13.00±0.02^{d}	15.00±0.00^{e}	11.00±0.01^{f}	13.00±0.01^{d}
Soil								
Azithromycin	23.00±0.00^{a}	25.00±0.02^{b}	24.00±0.02^{c}	28.00±0.02^{d}	24.00±0.02^{c}	28.00±0.02^{d}	26.00±0.01^{e}	30.00±0.00^{f}
Clarithromycin	10.00±0.01^{a}	13.00±0.00^{b}	12.00±0.01^{c}	15.00±0.00^{d}	9.00±0.01^{e}	11.00±0.00^{f}	11.00±0.00^{f}	13.00±0.01^{b}
Erythromycin	9.00±0.00^{a}	11.00±0.01^{b}	10.00±0.00^{c}	11.00±0.00^{b}	10.00±0.01^{c}	12.00±0.01^{d}	12.00±0.00^{e}	14.00±0.02^{f}
Drainage								
Azithromycin	29.00±0.00^{a}	32.00±0.01^{b}	28.00±0.00^{c}	30.00±0.01^{d}	30.00±0.01^{d}	34.00±0.02^{e}	31.00±0.01^{f}	33.00±0.01^{g}
Clarithromycin	10.00±0.01^{a}	12.00±0.02^{b}	12.00±0.01^{b}	14.00±0.01^{c}	14.00±0.01^{c}	16.00±0.01^{d}	11.00±0.00^{e}	15.00±0.01^{f}
Erythromycin	9.00±0.02^{a}	10.00±0.02^{b}	11.00±0.01^{c}	13.00±0.01^{d}	11.00±0.01^{c}	13.00±0.02^{d}	11.00±0.01^{c}	13.00±0.02^{d}
E. coli								
Well water								
Azithromycin	35.00±0.01^{a}	37.00±0.03^{b}	39.00±0.03^{c}	43.00±0.01^{d}	35.00±0.02^{a}	38.00±0.02^{e}	33.00±0.03^{f}	36.00±0.02^{g}
Clarithromycin	10.00±0.02^{a}	11.00±0.01^{b}	10.00±0.02^{a}	12.00±0.02^{c}	11.00±0.01^{b}	13.00±0.03^{d}	12.00±0.00^{c}	15.00±0.01^{e}
Erythromycin	13.00±0.01^{a}	15.00±0.01^{b}	10.00±0.02^{c}	13.00±0.01^{a}	11.00±0.01^{d}	15.00±0.02^{b}	11.00±0.01^{d}	12.00±0.02^{e}
Soil								
Azithromycin	33.00±0.00^{a}	35.00±0.01^{b}	24.00±0.02^{c}	28.00±0.02^{d}	23.00±0.03^{e}	25.00±0.01^{f}	26.00±0.02^{g}	29.00±0.01^{h}
Clarithromycin	11.00±0.01^{a}	12.00±0.02^{b}	12.00±0.00^{b}	14.00±0.02^{c}	10.00±0.02^{d}	13.00±0.01^{e}	13.00±0.00^{e}	16.00±0.02^{f}
Erythromycin	10.00±0.02^{a}	13.00±0.01^{b}	11.00±0.00^{c}	13.00±0.01^{b}	11.00±0.01^{c}	14.00±0.02^{d}	12.00±0.01^{e}	15.00±0.00^{f}
Drainage								
Azithromycin	29.00±0.01^{a}	33.00±0.01^{b}	25.00±0.01^{c}	28.00±0.03^{d}	27.00±0.01^{e}	33.00±0.00^{b}	30.00±0.00^{f}	35.00±0.02^{g}
Clarithromycin	9.00±0.01^{a}	10.00±0.02^{b}	11.00±0.02^{c}	13.00±0.02^{d}	11.00±0.02^{c}	13.00±0.02^{d}	12.00±0.02^{e}	15.00±0.02^{f}
Erythromycin	9.00±0.00^{a}	12.00±0.01^{b}	11.00±0.00^{c}	12.00±0.01^{b}	9.00±0.01^{a}	11.00±0.02^{c}	8.00±0.02^{d}	10.00±0.00^{e}
***Shigella* spp.**								
Well water								
Azithromycin	25.00±0.00^{a}	27.00±0.01^{b}	29.00±0.03^{c}	23.00±0.01^{d}	25.00±0.01^{a}	28.00±0.02^{e}	23.00±0.01^{d}	26.00±0.02^{e}
Clarithromycin	9.00±0.03^{a}	10.00±0.02^{b}	10.00±0.02^{b}	15.00±0.01^{c}	10.00±0.01^{b}	13.00±0.01^{d}	12.00±0.02^{e}	13.00±0.00^{d}
Erythromycin	8.00±0.02^{a}	9.00±0.01^{b}	10.00±0.02^{c}	11.00±0.00^{d}	7.00±0.04^{e}	9.00±0.03^{b}	7.00±0.03^{e}	10.00±0.02^{c}
Soil								
Azithromycin	33.00±0.01^{a}	36.00±0.02^{b}	27.00±0.03^{c}	28.00±0.02^{d}	29.00±0.03^{e}	32.00±0.02^{f}	36.00±0.02^{b}	39.00±0.01^{g}
Clarithromycin	10.00±0.01^{a}	11.00±0.02^{b}	13.00±0.01^{c}	15.00±0.03^{d}	10.00±0.01^{a}	13.00±0.00^{c}	13.00±0.02^{c}	15.00±0.01^{d}
Erythromycin	9.00±0.01^{a}	12.00±0.02^{b}	10.00±0.01^{c}	13.00±0.01^{d}	11.00±0.01^{e}	13.00±0.02^{d}	12.00±0.00^{b}	16.00±0.02^{e}
Drainage								
Azithromycin	28.00±0.02^{a}	33.00±0.01^{b}	23.00±0.01^{c}	27.00±0.02^{d}	24.00±0.03^{e}	26.00±0.01^{f}	19.00±0.00^{g}	23.00±0.02^{c}
Clarithromycin	9.00±0.03^{a}	11.00±0.01^{b}	8.00±0.02^{c}	10.00±0.02^{d}	11.00±0.01^{b}	14.00±0.02^{e}	12.00±0.04^{f}	14.00±0.02^{e}
Erythromycin	7.00±0.03^{a}	8.00±0.00^{b}	10.00±0.01^{c}	11.00±0.02^{d}	9.00±0.01^{e}	11.00±0.00^{d}	8.00±0.00^{b}	10.00±0.01^{c}

*average of quadruplet, Values with the same superscripts in the same row are not significantly different ($p < 0.05$).

Table 5. Susceptibility patterns of bacterial isolates in Fiyinfoluwa.

Macrolides/ Sources	Diameter of zone of inhibition (mm): Concentration of antibiotics (µg/mL)							
	June		July		August		September	
	250	500	250	500	250	500	250	500
***Salmonella* spp.**								
Well water								
Azithromycin	27.00±0.01^{a}	29.00±0.03^{b}	39.00±0.01^{c}	43.00±0.01^{d}	25.00±0.01^{e}	28.00±0.02^{f}	33.00±0.02^{g}	36.00±0.01^{h}
Clarithromycin	10.00±0.02^{a}	13.00±0.01^{b}	16.00±0.02^{c}	18.00±0.01^{d}	10.00±0.01^{a}	15.00±0.01^{e}	12.00±0.01^{f}	15.00±0.01^{e}
Erythromycin	7.00±0.00^{a}	9.00±0.01^{b}	10.00±0.02^{c}	14.00±0.02^{c}	7.00±0.01^{a}	10.00±0.01^{c}	9.00±0.01^{b}	10.00±0.01^{c}
Soil								
Azithromycin	23.00±0.01^{a}	26.00±0.01^{b}	27.00±0.01^{c}	32.00±0.01^{d}	29.00±0.01^{e}	32.00±0.01^{d}	26.00±0.01^{b}	29.00±0.01^{e}
Clarithromycin	7.00±0.03^{a}	9.00±0.01^{b}	10.00±0.02^{c}	11.00±0.02^{d}	13.00±0.03^{e}	15.00±0.01^{f}	11.00±0.02^{d}	14.00±0.01^{g}
Erythromycin	9.00±0.01^{a}	10.00±0.03^{b}	10.00±0.01^{b}	11.00±0.01^{c}	9.00±0.02^{a}	10.00±0.01^{b}	7.00±0.03^{d}	9.00±0.01^{a}
Drainage								
Azithromycin	38.00±0.01^{a}	40.00±0.02^{b}	33.00±0.00^{c}	37.00±0.01^{d}	34.00±0.00^{e}	36.00±0.01^{f}	29.00±0.01^{g}	33.00±0.02^{c}
Clarithromycin	6.00±0.04^{a}	10.00±0.00^{b}	8.00±0.02^{c}	10.00±0.01^{b}	10.00±0.02^{b}	13.00±0.01^{d}	11.00±0.00^{e}	13.00±0.01^{d}
Erythromycin	7.00±0.01^{a}	9.00±0.01^{b}	10.00±0.02^{c}	13.00±0.01^{d}	10.00±0.00^{c}	13.00±0.00^{d}	8.00±0.01^{e}	12.00±0.01^{f}
E. coli								
Well water								
Azithromycin	22.00±0.01^{a}	24.00±0.02^{b}	32.00±0.00^{c}	33.00±0.03^{d}	35.00±0.01^{e}	37.00±0.01^{f}	23.00±0.02^{g}	25.00±0.00^{h}
Clarithromycin	10.00±0.02^{a}	11.00±0.01^{b}	10.00±0.01^{a}	13.00±0.00^{c}	9.00±0.04^{d}	10.00±0.02^{a}	12.00±0.01^{e}	14.00±0.04^{f}
Erythromycin	10.00±0.00^{a}	13.00±0.01^{b}	9.00±0.00^{c}	11.00±0.02^{d}	9.00±0.01^{c}	12.00±0.02^{e}	11.00±0.01^{d}	13.00±0.02^{b}
Soil								
Azithromycin	33.00±0.02^{a}	36.00±0.00^{b}	24.00±0.02^{c}	26.00±0.01^{d}	25.00±0.01^{e}	29.00±0.00^{f}	36.00±0.02^{b}	37.00±0.02^{g}
Clarithromycin	8.00±0.01^{a}	10.00±0.01^{b}	9.00±0.03^{c}	11.00±0.01^{d}	10.00±0.01^{b}	13.00±0.02^{e}	7.00±0.01^{f}	9.00±0.02^{c}
Erythromycin	9.00±0.01^{a}	12.00±0.01^{b}	8.00±0.02^{c}	9.00±0.01^{a}	10.00±0.00^{d}	11.00±0.03^{e}	6.00±0.02^{f}	8.00±0.01^{c}
Drainage								
Azithromycin	28.00±0.00^{a}	30.00±0.01^{b}	23.00±0.01^{c}	25.00±0.01^{d}	24.00±0.01^{e}	26.00±0.01^{f}	21.00±0.01^{g}	23.00±0.02^{c}
Clarithromycin	5.00±0.03^{a}	7.00±0.01^{b}	8.00±0.02^{c}	11.00±0.01^{d}	11.00±0.02^{d}	14.00±0.01^{e}	9.00±0.00^{f}	10.00±0.01^{g}
Erythromycin	9.00±0.01^{a}	12.00±0.01^{b}	13.00±0.01^{c}	15.00±0.01^{d}	10.00±0.01^{e}	13.00±0.01^{c}	11.00±0.01^{f}	15.00±0.02^{d}
***Shigella* spp.**								
Well water								
Azithromycin	32.00±0.01^{a}	34.00±0.02^{b}	22.00±0.01^{c}	23.00±0.02^{d}	25.00±0.02^{e}	27.00±0.02^{f}	33.00±0.01^{g}	35.00±0.03^{h}
Clarithromycin	11.00±0.02^{a}	14.00±0.01^{b}	9.00±0.01^{c}	13.00±0.01^{d}	10.00±0.01^{e}	14.00±0.03^{b}	12.00±0.02^{f}	15.00±0.02^{g}
Erythromycin	6.00±0.01^{a}	9.00±0.02^{b}	7.00±0.02^{c}	10.00±0.03^{d}	8.00±0.01^{e}	11.00±0.01^{f}	9.00±0.01^{b}	10.00±0.01^{d}
Soil								
Azithromycin	31.00±0.01^{a}	33.00±0.02^{b}	30.00±0.01^{c}	36.00±0.01^{d}	35.00±0.01^{e}	37.00±0.02^{f}	26.00±0.00^{g}	27.00±0.01^{h}
Clarithromycin	10.00±0.02^{a}	13.00±0.01^{b}	9.00±0.01^{c}	11.00±0.01^{d}	8.00±0.01^{e}	12.00±0.00^{f}	8.00±0.00^{e}	10.00±0.02^{g}
Erythromycin	9.00±0.01^{a}	12.00±0.02^{b}	9.00±0.01^{a}	11.00±0.02^{c}	11.00±0.01^{c}	13.00±0.01^{d}	10.00±0.02^{e}	13.00±0.01^{f}
Drainage								
Azithromycin	29.00±0.00^{a}	31.00±0.02^{b}	33.00±0.01^{c}	35.00±0.02^{d}	34.00±0.02^{e}	36.00±0.00^{f}	31.00±0.01^{b}	33.00±0.03^{c}
Clarithromycin	9.00±0.00^{a}	11.00±0.01^{b}	8.00±0.02^{c}	12.00±0.00^{d}	13.00±0.04^{e}	15.00±0.01^{f}	9.00±0.01^{a}	10.00±0.01^{g}
Erythromycin	7.00±0.03^{a}	10.00±0.02^{b}	10.00±0.00^{b}	13.00±0.01^{c}	10.00±0.01^{b}	12.00±0.00^{d}	9.00±0.02^{e}	12.00±0.01^{d}

*average of quadruplet, *Values with the same superscripts in the same row are not significantly different ($p < 0.05$).*

Discussion

From this research work, it was observed with the various characteristics of identification that the samples contained three enteric bacteria viz; *E. coli*, *Salmonella* spp. and *Shigella* spp. This showed that *E. coli*, *Salmonella* spp. and *Shigella* spp. are the prevalent enteric bacteria in well-water, soil and drainage during the raining season in Erifun, Omisanjana, Ajilosun and Fiyinfoluwa areas of Ado-Ekiti metropolis.

Role of the environmental pollutant of surface water in the study area could explain the sources of this finding. Increase in flooding due to seasonal rainfall account for heavy pollution from improper fecal disposal both from roaming animal and probably from the human. The amount of microbial contamination could be increased in soil and drainage according to **Mahmodi and Javanmairdi** [13]. The presence of *E. coli*, *Salmonella* spp. or *Shigella* spp. in drinking water is a threat to human health. This bacterial can cause hemorrhagic, colitis, diarrhea, abdominal pain, bacillary dysentery and cholera as stated by **Ocepek et al.** [14].

The well-waters of the study area are contaminated with these three pathogens. Previous studies by **Bourne and Coetzee** [15] showed that water-borne diseases are responsible for about 20% of all death in children less than five years of age. This study correlates with the study of **Payment et al.** [16] where a statistically significant increase was reported in gastrointestinal illness in a population that drink contaminated water with a different type of coliform bacteria. Therefore, checking the load of contaminants in water supply and using the accurate technic for this infection is very important.

The susceptibility patterns of the bacteria isolated from the various samples (well-water, soil and drainage) revealed that *Salmonella* spp., *E. coil* and *Shigella* spp. were susceptible to azithromycin, clarithromycin and erythromycin but with a varying degree of susceptibility. Azithromycin showed the highest potency. A lot of enteric bacteria are known to show resistance to conventional antibiotics [17]. This resistance is due to various factors which can be ascribable to indiscriminate use of antibiotics on counter purchase of antibiotics not prescribed or abuse of antibiotics. The resultant effect of the above factors is the issue of resistance to most antibiotics. In this research work, it was observed that macrolide antibiotics have shown high potency on the enteric bacteria. The efficacy is due to difficulty in their accessibility.

These findings correlate with the report of **Byrugaba** [17] where it was found out to be potent. Azithromycin is an antibiotic useful for the treatment of several bacterial infections. This includes middle ear infections, strep throat, pneumonia, traveler's diarrhea, and certain other intestinal infections. It may also be used for some sexually transmitted infections including chlamydia and gonorrhea infections. Azithromycin has relatively broad but shallow antibacterial activity. It inhibits some Gram-positive bacteria, some Gram-negative bacteria, and many atypical bacteria [18]. Its mechanism of action is by preventing bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome, thus inhibiting the translation of mRNA. Nucleic acid synthesis is not affected [19].

Currently, azithromycin is recommended for the treatment of both shigellosis and invasive salmonellosis by the World Health Organization and the American Academy of Pediatrics [20, 21] and is increasingly used for the management of uncomplicated enteric fever [22,23]. However, clinical breakpoints for azithromycin and *Salmonella* have yet to be defined. Clinical breakpoints are necessary to detect emerging and changing patterns of resistance and to guide clinicians in the selection of effective antimicrobial therapy. The first step toward defining clinical breakpoints is to collect relevant data, including (i) pharmacodynamic data of the drug, (ii) pharmacological properties of the drug, (iii) clinical outcome data, and (iv) microbiological data, i.e., MIC data for the specific pathogen in question [12, 24].

Conclusion

The present study indicated that the well water, soil and drainage Erifun, Omisanjana, Ajilosun and Fiyinfoluwa areas of Ado-Ekiti were severely contaminated with *E. coli*, *Salmonella* spp., and *Shigella* spp. this is due to the lack of adequate sanitary measures. The isolated organisms were all susceptible to macrolide antibiotics with varying degrees of susceptibility.

Having discovered the presence of enteric bacteria in the locations researched and the consequences of their presence with subsequent susceptibility to macrolide antibiotics, I recommend that efforts should be put in place to discourage open defecation so that enteric infections should be prevented. However, first aid treatments using macrolides should be visited.

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