

DISTRIBUTION PATTERN AND ANTIBIOTICS SUSCEPTIBILITIES OF SOME AQUATIC SOIL MICROBES IN AKUNGBA-AKOKO COMMUNITY, NIGERIA

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ABSTRACT

Different soil samples were examined for the isolation of microorganisms and their susceptibility to different antibiotics. The places of collection include Akungba and Ayepe, Oka Akoko area. Area of collection includes; Alakuduru stream, Pond, Apex water, spring, Alakuduru stagnant water. The study shows that Alakuduru stagnant water has the highest colony forming unit of bacteria ranging between 5.44 cfu/g on MacConkey Agar and 5.18 cfu/g on chocolate Agar. Alakuduru stream has the lowest microbial load following incubation with total viable bacterial count of 4.76 cfu/gon chocolate Agar. A total number of fifteen isolates were cultured from all the five samples of soil sediment examined for occurrence of bacterial cells in the sediment. Following this study, one of the isolates; *Escherichia coli* which was almost prominent in all five samples was resistant to Amoxicillin antibiotics. This result may be an indication that *E. coli* has formed resistant strains against the antibiotic; hence the use of alternative therapy can be suggested. Furthermore, Of the 15 isolates, 10 were Gram negative, while 5 were Gram positive. The Gram staining reaction of the isolates indicated that 66.67% of the isolates were Gram negative while 33.33% were Gram positive.

Keywords: Antibiotics Susceptibilities, Microbes

INTRODUCTION

Soil is described as weathered rock combined with organic matter and nutrients. The complexity of soil as a habitat has been a challenge to understanding soil microbial ecology (Prescott *et al.*, 2008). Among the different microorganisms inhabiting in the soil, bacteria are the most abundant and predominant organisms. Some bacteria in the soil can be aerobic, facultative or anaerobic based on their oxygen requirements. The total viable counts in the soil generally range from 10^5g^{-1} in poor soil to 10^8g^{-1} in garden soil (Salle, 2002).

The bacteria in soil are primarily responsible for mineralization of organic matter, for fixation of nitrogen, for nitrification, for denitrification, and for some geochemically important processes such as sulphate reduction which cannot proceed in soil without the intervention of sulfate-reducing bacteria. Some bacteria especially copiotrophs (microorganisms that requires a nutrient-rich environment) often reside in microcolonies or biofilms on soil particles because it is through this they find optimum nutrition and other requirement needed for their existence. In a balanced soil, plants grow in an active and vibrant environment. The mineral content of the soil and its physical structure are important for their well-being, but it is the life in the earth that powers its cycles and provides its fertility. Without the activities of soil organisms, organic materials would accumulate and litter the soil surface and there would be no food for plants (Salle, 2002).

Bacteria are single-celled organisms and are the most numerous denizens of agriculture with populations ranging from 100million to 3 billion in a gram. They are capable of very rapid reproduction by binary fission, one bacterium is capable of producing 16 million more in just 24 hours. Bacteria lives in soil water including the film of moisture surrounding soil particles and some are able to swim by flagella. Majority of beneficial soil dwelling bacteria need oxygen (and are thus termed aerobic bacteria) whilst those that do not require air are referred to as anaerobic (and can be describe as the decomposer of protein

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and of most result in the breakdown of cohesion between tissues and liquification of most organs) (Olutiola *et al.*, 2000).

Aerobic bacterial are most active in a soil that is moist but not saturated, (as this will deprive aerobic bacteria of the air that they require), and neutral soil P^H and where there is plenty of food (CHO and micronutrient from organic matters available). Hostile conditions will not completely kill bacteria rather the bacteria will stop growing and get into a dormant stage and those individual with pro-adaptive mutation may compete better in the new conditions. Some Gram positive bacteria produces spore e.g. in order to wait for more favorable circumstances and Gram negative bacteria gets into a “non-culturable” stage.

Various groups of organisms that can be recovered from soil react differently to some chemical component in the environment and even to some antibiotics that can be administered in case of infection. Antibiotic sensitivity test is hence performed to determine the nature of these organisms. Antibiotic sensitivity is a term used to describe the susceptibility of bacteria to antibiotics. Antibiotic susceptibility testing is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo* (Finberg *et al.*, 2004).

These are the two major methods employed for estimation of soil microorganisms and they are; Agar plate method and direct microscopic method. According to Edgell (1960), a weighed sample of soil is mixed with a known volume of sterile water contained in a series of capped bottles, the sample is shaken vigorously to separate many organisms as possible from colloidal material surrounding the soil particle after the coarse particle have settled. Series of dilutions are prepared from the suspension, aliquote portion from each dilution are transferred to petridishes and incubate at 25°C for 2-14 days. The colonies are counted and the result expresses as the number of organisms per gram of soil. However, the disadvantages of this method include the following; obligate anaerobes did not grow in the presence of oxygen, autotrophic bacteria fail to multiply in organic media, non-symbiotic nitrogen-fixers grow to a limited extent.

Many cellulose decomposing organisms do not grow on commonly used media, and sulphate reducers grow in a medium containing mainly sulphate. Therefore, this method only represents count of the total microbial population of total microorganisms in the soil (Edgell, 1960).

The direct microscopic method can be intensified by the use of soil suspension transferred to a slide and spread out uniformly as described by Holding (1960). The slide is dried over a water bath; it is then covered with a solution of 1% Rose Bengal and 5% aqueous phenol. The bacteria take a deep pink or red color while the mineral constituent do not stain, some of the dead organic matter appear light pink, most stained light yellow or not at all, the slide is examined under calibrated oil immersion objective and the number of organisms per field is counted. This method also have some disadvantages as stated below; several slides must be prepared to take an average of total count and dead organisms are recorded, the organisms are not evenly distributed it may be difficult to separate soil particles from bacteria because they are both small in size. It has been established that the quantity of soil microflora can be remarkably influenced by growing plants. For example, a plant free soil may have 7% Gram negative bacteria compared to 20% in the rhizosphere (Holding, 1960; Casida, 1962).

The important function of soil microorganisms in the soil is to decompose various kinds of organic matter of plant and animal origin. This includes stable manures, plant stubbles, plant roots, organic fertilizers, and other related products. The decomposition of such compounds is as a result of activities of bacteria, molds, protozoa, and other organisms present in the soil. Each group selects certain constituent of the organic matter stable for synthesizing its own characteristic protoplasm (Alexander, 1997).

The organic compounds added to the soil as a result of biological action include various sugar, pentosans, cellulose, lignin, protein, amino acid, fats, waxes, tannins and pigments. These are decomposed further resulting in the libration of soluble organic and inorganic compounds. The inorganic compounds are notably ammonia and its salts. This may be utilized by plants as a source of nitrogen, organic materials especially stable and green manures are said to produce distinct effect upon soil process and plant growth (Foster and Raoult, 1994).

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The term "antibiotic" was coined by Selman Waksman (1942) to describe any substance produced by a microorganism that is antagonistic to the growth of other microorganisms in high dilution. This original definition excluded naturally occurring substances that kill bacteria but are not produced by microorganisms (such as gastric juice and hydrogen peroxide) and also excluded synthetic antibacterial compounds such as the sulphonamides. Many antibiotics are relatively small molecules with a molecular weight less than 2000Da. With advances in medicinal chemistry, most antibiotics are now semi synthetic-modified chemically from original compounds found in nature, as is the case with beta-lactams (which include the penicillins, produced by fungi in the genus *Penicillium*, the cephalosporins, and the carbapenems).

Some antibiotics are still produced and isolated from living organisms, such as the aminoglycosides, and others have been created through purely synthetic means: the sulphonamides, the quinolones, and the oxazolidinones. In addition to this origin-based classification into natural, semi synthetic, and synthetic, antibiotics may be divided into two broad groups according to their effect on microorganisms: Those that kill bacteria are bactericidal agents, whereas those that only impair bacterial growth are known as bacteriostatic agents. Many treatments for infections prior to the beginning of the twentieth century were based on medicinal folklore. Treatments for infection in ancient Chinese medicine using plants with antimicrobial properties were investigated over two centuries back (Lindblad, 2008).

The discovery of the natural antibiotics produced by microorganisms stemmed from earlier work on the observation of antibiosis between micro-organisms. Louis Pasteur observed that, "if we could intervene in the antagonism observed between some bacteria, it would offer 'perhaps the greatest hopes for therapeutics (Kingston, 2008). Synthetic antibiotic chemotherapy as a science and the story of antibiotic development began in Germany with Paul Ehrlich, a German medical scientist in the late 1880s (Paul and Clark, 1996; Calderon *et al.*, 2007).

Originally known as *antibiosis*, antibiotics were drugs which acted against bacteria. The term *antibiosis*, (which means "against life,") was introduced by the French bacteriologist Vuillemin as a descriptive name of the phenomenon exhibited by these drugs (Landsberg, 1949). (Antibiosis was first described in 1877 in bacteria when Louis Pasteur and Robert Koch observed that an airborne bacillus could inhibit the growth of *Bacillus anthracis* (Landsberg, 1949). These drugs were later renamed antibiotics by Selman Waksman (1942), an American microbiologist (Calderon *et al.*, 2007).

The assessment of the activity of an antibiotic is crucial to the successful outcome of antimicrobial therapy. Non-microbiological factors such as host defence mechanisms, the location of an infection, the underlying disease as well as the intrinsic pharmacokinetic and pharmacodynamic properties of the antibiotic (Pankey and Sabath, 2004). Fundamentally, antibiotics are classified as either having lethal (bactericidal) action against bacteria or are bacteriostatic, preventing bacterial growth.

Antibiotics are widely used in the prevention and treatment of infectious diseases. Chemical substances such as penicillin, streptomycin, chloramphenicol, and tetracycline produced by various microorganisms or made synthetically are capable of destroying or inhibiting the growth of microorganisms especially bacteria. They inhibit pathogens by interfering with essential intracellular processes, including the synthesis of bacterial proteins. Antibiotics do not kill viruses and are not effective in treating viral infections (Kingston, 2008).

The sensitivity of an isolate to a particular antibiotic is measured by establishing the Minimum Inhibitory Concentration (MIC) or breakpoint, this is the lowest concentration (conventionally tested in doubling dilutions) of antibiotic at which an isolate cannot produce visible growth after overnight incubation. Different types of antibiotics that are considered for this purpose are penicillin, Cloxacillin, Tetracycline, Streptomycin, Nitrofurantoin, Nalidixic acid, Augmentin, Chloramphenicol, Ciprofloxacin, among others. Nitrofurantoin is an antibiotic which is marketed under the following brand names; Furadantin, Macrobid, Macroclantin, Nitrofur Mac, Nitro Macro, Nifty-SR, Martifur-MR, Martifur-100 (in India), Urantoin, and Uvamin (in Middle East). It is usually used in treating urinary tract infection. Like many other drugs, it is often used against *E. coli*. Resistance to other antibiotics has led to increased interest in this agent (Garau, 2008; Carone *et al.*, 2014). It is sometimes described as being appropriate to use in pregnant patients

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(along with other agents such as sulfisoxazole or cephalexin) (Lee *et al.*, 2008). Other antibiotic to be considered is Nalidixic acid (trade names Nevigramon, Neggram, Wintomylon and WIN 18,320) which is the first of the synthetic quinolone antibiotics. Synthetic quinolone antibiotics were discovered by George Leshner and coworkers as a byproduct of chloroquine manufacture in the 1960s (Emmerson *et al.*, 2003).

Amoxicillin (INN), formerly amoxicillin (BAN), and abbreviated amox, is a moderate-spectrum, bacteriolytic, β -lactam antibiotic used to treat bacterial infections such as skin infections, urinary tract infections, *salmonella*, lyme disease, and Chlamydia infections (Thornhill *et al.*, 2011) caused by susceptible microorganisms.

Ampicillin is a beta-lactam antibiotic that has been used extensively to treat bacterial infections since 1961. Until the introduction of ampicillin by the British company Beecham, penicillin therapies had only been effective against Gram-positive organisms such as *staphylococci* and *streptococci*. Ampicillin (originally branded as 'Penbritin') also demonstrated activity against Gram-negative organisms such as *Haemophilus influenzae*, coliforms and *Proteus* spp. Ampicillin was the first of a number of so-called broad spectrum penicillins subsequently introduced by Beecham. Ampicillin is part of the aminopenicillin family and is roughly equivalent to its successor, amoxicillin in terms of spectrum and level of activity (Kasten and Reski, 1997).

Chloramphenicol is a bacteriostatic antimicrobial. It is considered a prototypical broad-spectrum antibiotic, alongside the tetracyclines. Chloramphenicol is effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. Due to resistance and safety concerns, it is no longer a first-line agent for any indication in developed nations, although it is sometimes used topically for eye infections. Nevertheless, the global problem of advancing bacterial resistance to newer drugs has led to renewed interest in its use (Falagas *et al.*, 2008). In low-income countries, chloramphenicol is still widely used because it is inexpensive and readily available.

Ciprofloxacin is a synthetic chemotherapeutic antibiotic of the fluoroquinolone drug class (Nelson *et al.*, 2007; Kawahara, 1998). It is a second-generation fluoroquinolone antibacterial. Ciprofloxacin is used to treat a number of infections including: infections of bones and joints, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, chancroid, among others (Alexander *et al.*, 2004). Erythromycin is a macrolide antibiotic that has an antimicrobial spectrum similar to or slightly wider than that of penicillin, and is often used for people who have an allergy to penicillin. For respiratory tract infections, it has better coverage of atypical organisms, including mycoplasma and Legionellosis. Gentamicin is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by Gram-negative organisms (Moulds and Jeyasingham, 2010).

Objectives of this study are to isolate and identify bacterial species obtained from soil samples used for this study. Similarly, the distribution pattern of aquatic soil microbes in Akungba-Akoko community, Nigeria was determined. Therefore, this study will help to assist the clinicians and health care management on the control measures for the treatment of infections.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from five different places in Akungba Township. About 5g-10g of the soil samples were collected using a sterile universal bottle for each of the samples collected. Each of the samples was coded. For instance soil sample collected from Alakuduru stream was coded ASM, while the stagnant was coded AST and other locations from where samples were collected are coded P for pond, S for spring, A for Apex.

Sterilization Techniques

The materials used for this study including glassware such as the petridishes, pipettes, test tubes, beakers, conical flasks and McCartney bottles were thoroughly washed with detergents and rinsed with clean water and were subsequently sterilized under aseptic conditions. The inoculating loop, wire loop were sterilized by flaming in Bunsen burner until red hot, the surface of the working bench was also sterilized by

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swabbing with antiseptic solution of 95% ethanol. Petridishes were arranged inside the canister before placing inside the oven. The glassware was sterilized at 160°C for 1 hour.

Media Used

The culture media generally used for this study includes, Nutrient Agar (for sub-culturing), MacConkey Agar (coliform count), chocolate Agar, Muller-Hinton Agar (for antibiotics sensitivity testing) as well as Peptone water (for sugar fermentation). The media were prepared in accordance to the manufacturer's instruction or standard methods for examination of soil samples. The compounds were suspended in appropriate amount of distilled water and plugged with cotton wool and subsequently covered with aluminum foil before autoclaving.

Identification and Preservation of Bacterial Isolates

1g of each sample collected were serially diluted and inoculated into sterile petridishes for incubation at 37°C for a period of 24-48 hours using appropriate media until a reasonable growth was seen. Unaided eyes were used to observe the cultural characteristics of the bacterial colonies formed on the plates. These characteristics include; shape, edge, elevation, surface, colour. Each of the isolates was streaked on the surface of separate agar slants aseptically prepared and incubated at 37°C for 24 hours. The isolates were kept in refrigerator at 4°C to serve as stock culture for subsequent test during identification. Isolates were identified using standard biochemical tests including Gram's staining technique and fermentation of sugars.

Antibiotics Sensitivity Test

This test was carried out to determine the susceptibility of the bacterial isolates to antibiotics. For this purpose, Gram negative and Gram positive antibiotic multidiscs were used. The Gram negative antibiotic disc contained ten different antibacterial agents which are; Augmentin (30µg), Nitrofurantoin (200µg), Gentamicin (10µg), Cotrimoxazole (25µg), Ofloxacin (5µg), Amoxicillin (25µg), Ciprofloxacin (10µg), Tetracycline (30µg). While the Gram positive antibiotics disc contained ten different antibacterial agents which are; Amoxicillin (25µg), Ofloxacin (5µg), Streptomycin (10µg), Chloramphenicol (30µg), Gentamicin (10µg), Erythromycin (5µg), Ciprofloxacin (10µg), Cotrimoxazole (25µg). Muller-Hilton agar was prepared under aseptic conditions and poured into plates. These plates were allowed to solidify at 45°C. A flamed inoculating loop was then used to inoculate the media with a 24hour old culture to ensure adequate results. The inoculum was then streaked all over the plate to ensure maximum distribution. A pair of sterile flamed forceps was used to pick the sensitivity discs and it was placed on the Petri dishes, care was taken to ensure that the discs stood at the centre of the plates. This procedure was carried out for all the organisms, after which the plates were inverted and incubated at 37°C for 24 hours. All samples incubated were then observed for inhibition zones by the after 24hr and the results were recorded.

Interpretation and Reading of Antibiotic Sensitivity Test Result

The *in vitro* antibiotic susceptibility testing of bacterial isolates was performed using the standardized disc agar diffusion methods of the CLSI, (2012). After incubation, clear zones of growth of incubation around each disc showed the relative susceptibility of each isolate towards the various antibiotics. The diameter of the zones of inhibition was measured by means of calibrated ruler in millimeter from the underside of the plates. Isolates were scored as either sensitive if there was growth inhibition or resistant if there was no growth inhibition. Clear zones of inhibition that were less than or equal to 6mm were taken as resistant and zones of inhibition more than 6mm were recorded as sensitive.

Fermentation of Sugars

Phenol red (0.1g), sodium chloride (1.0g), fermentable sugars (1.0g) was weighed into a conical flask containing 100ml of nutrient broth. The mixture was swirled so that all components in it can dissolve. 5ml of the preparation was dispensed into test tubes containing inverted Durham tubes. The tubes were covered with cotton wool and aluminum foil and were sterilized using autoclave at temperature of 121°C for 15 minutes. Sugars used include; glucose, mannitol, lactose, sucrose. 1% of the respective sugars were added to constitute the medium.

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All test tubes were inoculated with respective organism aseptically and incubated at 37°C; results were examined daily for 7 days. A change in the colour of indicator (phenol red) to yellow indicates utilization of sugars (acid production) and gas production was detected by the accumulation of the gas produced in the Durham tubes. No gas can be produced without acid formation but acid may be produced without gas being produced. Uninoculated test tubes were used as control for each sugar test.

RESULTS AND DISCUSSION

Result

Five different soil samples were examined for the isolation of different microorganisms and their susceptibility to different antibiotics. The places of collection include Akungba and Ayepe, Oka Akoko area.

Area of collection includes; Alakuduru stream, Pond, Apex water, spring, Alakuduru stagnant water. Identification of the isolates were based on their cultural, morphological and biochemical characteristics. A total number of fifteen isolates were cultured from all the five samples of soil sediment examined for occurrence of bacterial cells in the sediment. Of the 15 isolates, 10 were Gram negative, while 5 were Gram positive.

The morphological characteristics observed from these isolates varies and include; cream, convex, pink, gold, green, mucoid, non-mucoid, circular, dry, opaque, red, swarm and concave.

The Gram staining reaction of the isolates indicated that 66.67% of the isolates were Gram negative while 33.33% were Gram positive.

Table 1: Morphological Characteristics of Isolates

Sample Code	Gram Stain	Shape	Morphology on Mac	Morphology on Choc	Suspected Organisms
ASM 1	-	Short rod	Opaque, pink, round, convex, non-mucoid	Circular, round, non-mucoid colonies	<i>Escherichia</i> , <i>Shigella</i>
ASM 2	+	Cocci in cluster	Colourless, flat, non-mucoid	Creamy, convex, smooth flat	<i>Staphylococcus</i>
	+	Cocci in small chains	Dry pinkish colonies	Pinkish, mucoid, elevated edge	<i>Streptococcus</i>
S 1	-	Short rod	Red, circular, convex, non-mucoid	Flat, colourless, swarm, non-mucoid	<i>Proteus</i>
S 2	-	Short rod	Opaque, pink, circular, non-mucoid	Circular, smooth, flat, pinkish red convex	<i>Salmonella</i>
A 1	+	Cocci in cluster	Creamy, round convex, non-mucoid	Creamy, convex, smooth, non-mucoid	<i>Staphylococcus</i>
A 2	-	Short rod	Red, circular, convex, non-mucoid	Flat, colourless, swarm	<i>Escherichia</i> , <i>Proteus</i>
AST 1	-	Rod	Pink, circular large colony	Red circular non-mucoid colonies	<i>Klebsiella</i>
AST 2	-	Short encapsulated rod	Pinkish mucoid colony	Green concave flat mucoid	<i>Pseudomonas</i>
P 1	-	Rod	Pinkish large circular, convex, flat, swarm	Circular red, non-mucoid colonies	<i>Klebsiella</i> , <i>Escherichia</i>
P 2	+	Rod	Dry pink surface colonies	Creamy flat colonies	<i>Pseudomonas</i>

Keys: ASM: Alakuduru Stream; A: Apex Stream; AST: Alakuduru Stagnant Water; P: Pond; S: Spring Water; -: Gram Negative; +: Gram Positive

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Table 2: Biochemical Characteristics and Identification of the Isolates

S/ NO	Motility	Indole	Urease	Voges-Proskauer	Oxidase	Citrate	Catalase	Coagulase	Sucrose	Glucose	Lactose	Mannitol	Gram stain	Morphology	Probable Organism
1	+	-	-	-	-	-	+	-	-	Ag	Ag	-	-	Rods	<i>Escherichia</i>
2	-	+	slow	+	-	+	-	ND	+	Ag	Ag	+	-	Rods	<i>Klebsiella</i>
3	+	-	+	ND	-	+	-	ND	Ag	A	Ag	-	-	Rods	<i>Proteus</i>
4	+	-	-	-	-	+	+	-	-	+	-	+	-	Rods	<i>Salmonella</i>
5	+	-	-	-	+	+	+	-	-	Ag	Ag	-	-	Rods	<i>Pseudomonas</i>
6	-	-	-	-	-	-	+	+	-	+	-	+	+	Cocci in clusters	<i>Staphylococcus</i>
7	-	-	+	-	-	-	-	-	-	-	+	-	+	Rods	<i>Clostridium</i>
8	-	-	-	-	-	-	-	-	-	+	-	-	-	Rods	<i>Shigella</i>
9	+	-	+	ND	-	+	-	ND	Ag	A	Ag	-	-	Rods	<i>Proteus</i>
10	+	+	-	-	-	-	+	-	-	Ag	Ag	+	-	Rods	<i>Escherichia</i>
11	-	-	-	-	-	-	+	+	A	+	-	+	+	Cocci in clusters	<i>Staphylococcus</i>
12	-	-	slow	+	-	+	-	ND	+	Ag	Ag	+	-	Rods	<i>Klebsiella</i>
13	+	-	-	ND	-	-	-	-	A	A	Ag	+	+	Rods	<i>Bacillus</i>
14	-	-	-	-	-	-	-	-	A	A	A	+	+	Cocci in chains	<i>Streptococcus</i>
15	+	+	-	-	-	-	+	-	-	Ag	Ag	+	-	Rods	<i>Escherichia</i>

Keys: +: Positive; -: Negative; ND: Not Determined; A: Acid Fermentation; Ag: Acid and Gas Production

Table 3: Incidence of Occurrence of Bacterial Isolates in Percentage

Isolates	No of Occurrence	Incidence of Occurrence	Percentage (%)
<i>Escherichia</i>	3	Pond, Alakuduru stream, Apex	20%
<i>Klebsiella</i>	2	Pond, Alakuduru stagnant water	13.33%
<i>Staphylococcus</i>	2	Alakuduru stream, Apex	13.33%
<i>Proteus</i>	2	Spring water, Apex	13.33%
<i>Salmonella</i>	1	Spring	6.667%
<i>Shigella</i>	1	Alakuduru stream	6.667%
<i>Pseudomonas</i>	1	Alakuduru stagnant water	6.667%
<i>Streptococcus</i>	1	Alakuduru stream	6.667%
<i>Bacillus</i>	1	Pond	6.667%
<i>Clostridium</i>	1	Pond	6.667%

NB: The percentage of occurrence is the ratio of the number of isolate to the total number of the isolates, multiplied by 100%

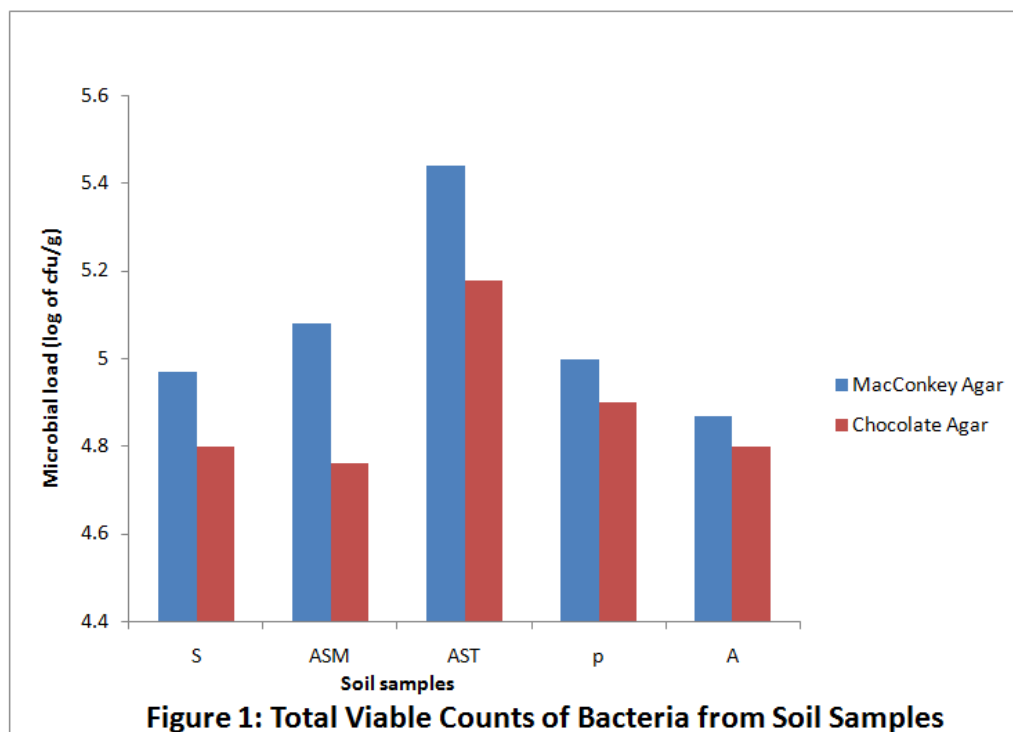


Figure 1: Total Viable Counts of Bacteria from Soil Samples

Keys: S – Spring; ASM – Alakuduru Stream; AST – Alakuduru Stagnant Water; P – Pond; A– Apex Stream

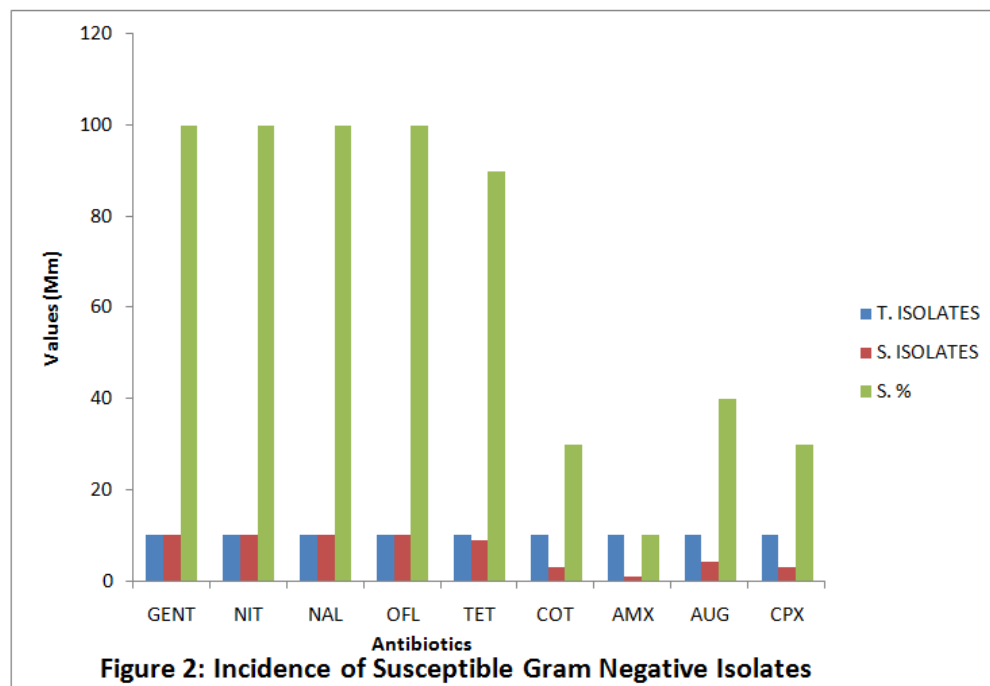


Figure 2: Incidence of Susceptible Gram Negative Isolates

Keys: CPX: Ciprofloxacin; NIT: Nitrofurantoin; NAL: Nalidixic Acid; OFL: Ofloxacin; TET: Tetracycline; COT: Cotrimoxazole; AMX: Amoxicillin; AUG: Augmentin; GENT: Gentamicin; T. Isolates: Tested Isolates; S. Isolates: Susceptible Isolates; S. %: Percentage of Susceptibility

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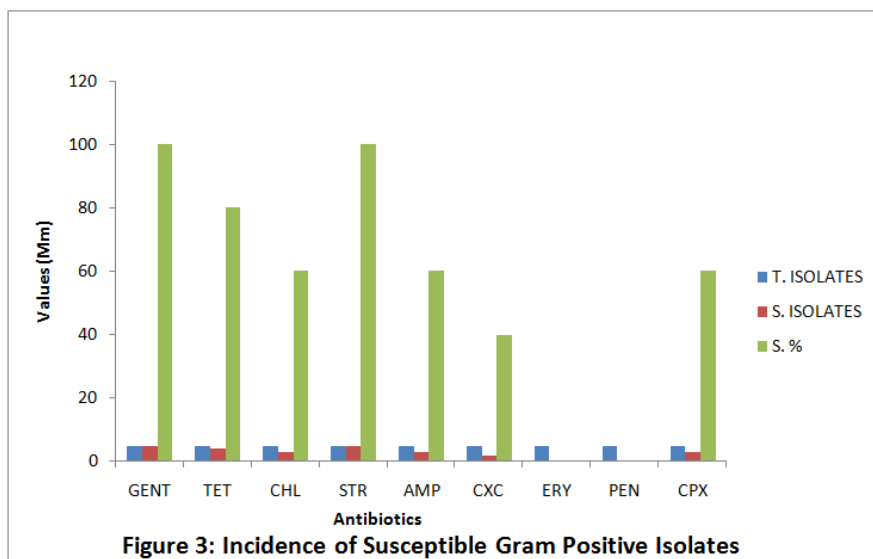


Figure 3: Incidence of Susceptible Gram Positive Isolates

Keys: TET: Tetracycline; AMP: Ampicillin; CPX: Ciprofloxacin; STR: Streptomycin; CHL: Chloramphenicol; PEN: Penicillin; CXC: Cloxacillin; ERY: Erythromycin; GENT: Gentamicin; T. Isolates: Tested Isolates; S. Isolates: Susceptible Isolates; S. %: Percentage of Susceptibility

Discussion

Various organisms were isolated from five different sources in Akungba-Akoko community, Nigeria. Result of the study in Figure1 shows that Alakuduru stagnant water has the highest bacteria count of 5.44 cfu/g. The factor responsible for this may be due to the Stagnancy of the water body, moreover the nutrients in this source remains without being washed away thus increasing the metabolic activity of the microorganisms situated around this area leading to the proliferation of the microbial population. Alakuduru stream has the lowest microbial load following incubation with total bacterial count of 4.76 cfu/g. The Alakuduru stream source is a flowing stream thus allowing the movement of microorganisms and nutrients into and out of the water. This result was similar to Rabah *et al.*, (2010) while accessing soil contaminated with abattoir effluent. The presence of *E.coli* may be attributed to the high load of animal excreta and sewage effluents (Ezeronye and Ubalua, 2005).

Tables 1 and 2 show the morphological characteristics and biochemical reactions of the isolates. Some of the isolates from Alakuduru stream differ from those isolated from Alakuduru stagnant water based on their morphological appearances and Gram staining techniques which distinguish Gram positive microbes from Gram negative ones. The majority of the isolates from both Alakuduru stream and stagnant water were Gram negatives and following the biochemical reaction, they were mostly confirmed to be enteric microorganisms which may be introduced into the water from human and animal faeces, and can cause water borne diseases (Ezeronye and Ubalua, 2005).

Both Gram positive and Gram negative bacteria were isolated from the pond source whereas only Gram negative microorganisms were isolated from the spring water and Apex stream. Similarly, Table 3 shows incidence of occurrence of bacterial isolates form sampled sources, which helps to clarify the distribution pattern of the identified microbes in nature.

The result in Figure 2 revealed that bacterial isolates including *E. coli* isolated from pond, Alakuduru stream and Apex were all resistant to Amoxicillin antibiotics, and this may be an indication that *E. coli* has form resistant strains against the antibiotic, hence the use of alternative therapy can be suggested. This same organism was susceptible to GENT, NIT, OFL and TET with *E. coli* from pond having the highest sensitivity reaction to OFL and *E. coli* from Alakuduru stream has the lowest diameter of zone of inhibition to TET. Only *E. coli* isolated from Alakuduru stream was resistant to COT, AMX, and AUG while *E. coli* isolated from Apex was resistant only to AMX.

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Based on the sources, study revealed that both *E. coli* and *Shigella* isolated from Alakuduru stream were resistant to AMX, also *E. coli* and *Klebsiella* from pond were resistant to AMX. Riedal *et al.*, (1999) concluded that antibiotics do not enter the lactamase ring of microorganisms, until recently *E. coli* isolates were uniformly sensitive to antimicrobial agents. In Figure 3, it was indicated that all the Gram positive isolates were resistant to Erythromycin (ERY) and Penicillin (PEN). *Staphylococcus aureus* isolated from both Alakuduru stream and Alakuduru stagnant water were resistant to ciprofloxacin. A report was given (Allen *et al.*, 1999) that selective antimicrobial pressure and multiple admissions to hospitals were among the risk factors associated with antimicrobial resistant to antibiotics.

In conclusion, it is crucial to recognize that drug therapy is not a simple matter, drugs may be administered in several ways, and they do not always spread rapidly throughout the body or immediately kill all invading pathogens. Complex factors influence the effectiveness of drugs and these includes, ability of the drug to be able to reach the actual site of infection, susceptibility of the pathogens to the drugs and ability of the chemotherapeutic agent to exceed the pathogen's MIC value.

Since most of the microorganisms isolated from the water sediment in this study were enteric microorganisms which find their ways into the water body through various means which include; contamination from human and animal feces, pollution by dumping of refuse into streams, hence proper hygiene and sanitation must be observed in our environment in order to reduce the existence of the microbial population in the community.

It should be noted that the administration of antibiotics must not be done prior to the detection of causative agent of a particular illness because abusive usage of these antimicrobial agents may increase the level of resistance of these bacteria to the antibiotics thereby rendering such drugs ineffective when used for treatment.

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