

RESEARCHS / INVESTIGACIÓN

Control of bacterial pathogens isolated from water using Actinomycetes extracts at Egerton University, Kenya

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Abstract: Diseases are the worst enemy to man currently. This study was aimed at isolating pathogenic bacteria from water obtained from shallow wells in Dundori Kenya. Also, the study aimed at testing the isolates for sensitivity to antibiotic metabolites previously extracted from Actinomycetes isolates from soils of Egerton University. Water samples were collected from shallow wells randomly selected from Dundori and abbreviated as A, B, C, D, and E. Bacterial pathogens were isolated from the water samples using the membrane filtration technique. The isolates were characterized using biochemical means. Anti-microbial sensitivity testing was carried out using Kirby Bauer disk diffusion method. Data analysis was carried out using the Statistical Package for Social Sciences (SPSS). Comparison of means was carried out using one way ANOVA. Shallow wells B, D and E were highly contaminated with pathogenic bacteria. Biochemical characterization of the isolates indicated that the most common isolates were *Vibrio cholera*, *Klebsiella pneumoniae*, *Proteus* sp., *Escherichia coli*, and *Staphylococcus aureus*. There was no significant difference between the zones of inhibition produced by the antibiotic metabolites ($F=2.149$ $P=1340$) when tested against the test isolates. There were no significant differences between the MIC's of the antibiotic metabolites on the bacterial pathogens ($F=2.01$ $P=0.15$). Water from some shallow wells in Dundori is highly contaminated with *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus* sp., *Vibrio cholerae* and *Staphylococcus aureus*. The pathogens can effectively be controlled using antibiotics from the Actinomycetes. There is a need to sensitize the residents of the study area on ways of preventing seepage of contaminants into the shallow wells.

Keywords: Bacteria, Egerton, Control, Pathogens, Isolation, Water.

Introduction

Water from wells is considered contaminated or unsafe for drinking when tested for toxic chemicals or pathogenic microorganisms ¹. The primary source of contamination by microorganisms could be fecal waste from warm-blooded animals including humans. Different pathogenic bacteria cause enteric diseases to humans while others are non-pathogenic ². The water could also support the growth of protozoan and viral pathogens; hence water test for each pathogen would be extremely expensive and tedious ³. Water is considered unsafe for human consumption when it has pathogenic microorganisms. Water test for bacteriological safety depends on microbiologists' ability to detect coliform bacteria in the wells ⁴. *Escherichia coli* is the abundant bacteria in the test since it lives longer in water than other intestinal bacteria ⁵

Presence of pathogenic microorganisms in water sources may arise from contamination with fecal material, wastes from vegetation and animal dung resulting in growth of coliform bacteria ⁶. As a result, it becomes economically expensive for the communities to dig new wells with a low level of contamination ⁷. People who take contaminated water visit health facilities due to stomachache, diarrhea and flu-like symptoms. According to GOK (2014) ⁸ health statistics shows that pupils in Dundori have a high absenteeism rate and the cost of living in the area is rising at a very high rate.

In developing countries, contamination of water resources is indicated by the presence of enteric pathogens ⁹. However, rural communities use water disinfection methods such as chlorination, coagulation, sedimentation, and boiling ¹⁰. Some of these methods become impractical due to the high cost of

the required equipment and low availability of chemical coagulants ¹¹. Hundreds of people in Dundori area depend on both private and public wells which are infested with coliform bacteria. As a result, there is a need to control and improve the health safety of the water to levels that are considered safe for human consumption ¹². The area is facing a challenge on how to offer more people access to clean and safe drinking water ¹³.

Actinomycetes have been used as potential sources of many bioactive compounds which have diverse clinical effects and essential applications in human medicine ¹⁴. Antibiotic metabolites screened from Actinomycetes isolated from soils of Egerton University may be a remedy to contamination of water with bacterial pathogens ¹⁵. The aims of this study were to isolate pathogenic bacteria from shallow wells of Dundori and carry out the sensitivity test of the isolates to antibiotics previously screened from Actinomycetes isolated from the soils of Egerton University.

Materials and methods

Study area

The study was carried out in Dundori ward with a population of 43,482. The geographical area is sloppy hence the risks of contamination of shallow wells with bacteria. According to Amata *et al.* (2014) ¹⁶, Dundori region has an average temperature of 17°C 63°F and lies at latitude 0.27 and longitude 36.1.

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The Actinomycetes antibiotic metabolites

Four antibiotic metabolites coded EU30, EU37, EU41 and EU 154 had previously been extracted from Actinomycetes isolated from soils of Egerton University were used in this study. The Actinomycetes were grown in Luria Bertani broth for 7 days. The metabolites were extracted using ethyl acetate ¹⁷.

Water sampling

Five shallow wells coded A, B, C, D, and E were randomly selected from the study area. A total of 255 water samples were collected using sterile sample bottles. The sample size was determined using the formula by Waithaka *et al.* (2015) 18.

$$n = \frac{Z^2pqD}{d^2}$$

Where; n = sample size, p = anticipated prevalence which was 21% (0.21) in this study, q = failure which was calculated as 100-21 giving 79% (0.79), Z = is the appropriate value from the normal distribution for the desired confidence level which was 1.96 in this study, d = allowable error (0.05) and D = design effect. Based on a confidence interval of 95%, allowable error of 5% and a Z value of 1.96 the sample size was;

$$n = \frac{1.96^2(0.21 - 0.79)}{0.05^2} = 254.928 = 255$$

When collecting the samples, an empty ½ inch was left in the bottle for air space. The samples were transported to Egerton University Department of Biological Sciences Laboratories in iced containers and stored under refrigerated conditions at -4°C awaiting further processing.

Isolation of bacterial pathogens

One hundred milliliters water samples were filtered using membrane filters (Whatman GmbH, Germany), pore size 0.45µm, 47 mm diameter 19. The membrane filters were aseptically placed on sterile nutrient agar in nutrient agar. Incubation was carried out at 37°C for 24h. Enumeration of bacteria was carried out with the help of a colony counter.

Biochemical characterization

Biochemical identification of the isolates was carried out using API 20E strip kit (bioMérieux®, Inc., France). The reagents used included API NaCl 0.85 % medium, API 20 E reagent kit, Zn reagent, oxidase, mineral oil, and API 20E Analytical Profile Index 20. The strips were prepared by the use of an incubation box (tray and lid). In this preparation, 5ml of distilled water was distributed into the honey-combed wells of the tray to create a humid atmosphere ²¹. Inocula of pure isolates were, emulsified in 5ml of normal saline 0.85% NaCl to achieve a homogeneous bacterial suspension. Anaerobiosis in the

tests arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), H₂S production (H₂S), Urease (URE), citrate utilization (CIT), fermentation/oxidation of glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), melibiose (MEL) and arabinose (ARA), gelatinase (GEL), tryptophane deaminase (TDA), fermentation/oxidation of sugars sucrose (SAC) and amygdaline (AMY), Indol (IND) and acetoin (VP) production was maintained by overlaying with mineral oil. The incubation box was closed and incubated at a temperature 37°C for 24h as described by the manufacturer, and the results were determined according to API 20E catalog. The microorganisms were further identified using colony morphology, Gram staining and motility test ²².

Antimicrobial sensitivity testing

The antimicrobial susceptibility testing was carried out by use of Kirby Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) ²². Sterile wire loop was used to pick 3 colonies of each *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus Sp.*, *Vibrio cholera* and *Staphylococcus aureus*. Emulsification in 3 ml of sterile physiological saline was carried out. Standardization of the suspended colonies was performed by diluting the normal saline suspension until the turbidity matched 0.5 McFarland standards. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 20ml of Mueller-Hinton agar in sterile Petri plates. Discs (ABTEK BIOLOGICAL LTD., UK) 8mm in diameter were aseptically dipped into each of the antibiotic metabolites. The discs were air dried and placed on the agar using sterile forceps. The plates were incubated aerobically at 37°C for 24h. The zones of inhibition were measured in millimeters.

Statistical Analysis

Data obtained was presented in the form of tables. ANOVA was used to compare the means of viable cells in well A, B, C, D and E, zones of inhibition of the bacterial pathogens by the Actinomycetes crude extracts and minimum inhibitory concentration of the crude extracts.

Results

Number of viable cells obtained from the shallow wells from Dundori

There were no viable cells isolated from well A and well C (Table 1). The number of viable cells isolated from well B varied from 25 to 46 per ml of water. However, in well D the range of viable isolated cells was 227 to 325. In well E, the viable cells ranged from 680 to 1020 per ml of water sample. There was a significant difference in the number of viable isolated cells in well A, B, C and D (F=154.265 P=0.0009).

S. No	Well				
	A	B	C	D	E
1	0	40	0	289	970
2	0	25	0	300	680
3	0	32	0	322	795
4	0	46	0	227	1020
5	0	30	0	325	1000

Table 1. Number of viable cells (CFU/ml) isolated from well A, B, C, D, and E

Biochemical characteristics of the isolates

All the isolates apart from *Vibrio cholerae* were positive for catalase test. Also, the isolates were positive for the indole test except for *Klebsiella pneumoniae* and *Proteus sp* (Table 2). All the isolates were positive for Voges proskauer test except *Escherichia coli* and *Proteus sp*. However, the isolates were positive for citrate utilization except *Escherichia coli* and *Vibrio cholerae*.

On the other hand, all the isolates tested negative for methyl red except *Proteus sp*. and *Vibrio cholerae*. All the isolates tested negative for coagulase test except *Staphylococcus aureus*. Also, the isolates tested negative for oxidase test except for *Staphylococcus aureus*.

Zones inhibition of the isolates by the Actinomyces metabolites

The zones of inhibition of *K. pneumoniae* varied from 21±0.2mm in EU 154 to 23±0.3mm in EU 30 (Table 3). In *E. coli*, the zones of inhibition ranged between 18±0.3mm in EU 37 to 23±0.2mm in EU 41. Besides, the zones of inhibition in *Proteus sp.* ranged between 19±0.3mm in PAN 154 to 23±0.2mm in EU 30. However, in *V. cholera* the zones of inhibition varied

from 18±0.2mm in EU 154 to 25±0.3mm in EU41. In *S. aureus*, the zone of inhibition ranged between 22±0.3mm in EU 154 to 27±0.2 in EU 41. There was no significant difference between the zones of inhibition produced by the antibiotic metabolites (F=2.149 P=1340) when tested against the test isolates. The zones of inhibition were indicated by clearing around the disk having the antibiotic metabolites (Figure 1).

Minimum inhibitory concentration (MIC) of the antibiotic metabolites

The minimum inhibitory concentration (MIC) in *K. pneumoniae* varied between 0.23±0.03mg/ml in EU 41 to 0.25±0.01mg/ml in EU 30 (Table 4). In *Escherichia coli*, the MIC varied from 0.19±0.01mg/ml in EU 37 to 0.24±0.02mg/ml in EU 30. However, in *Proteus sp.* the MIC ranged between 0.18±0.01mg/ml in EU 41 and EU 154 to 0.25±0.02mg/ml EU 30. In *V. cholera*, the MIC varied from 0.17±0.02mg/ml in EU 41 to 0.25±0.02mg in EU 41. In addition, in *S. aureus*, the MIC varied from 0.23±0.01mg/ml in EU 41 to 0.25±0.01mg/ml in EU 30. There was no significant differences between the MIC's of the antibiotic metabolites on the bacterial pathogens (F=2.01 P=0.15).

Indole	M. Red	V. Proskauer	C.utilization	Coagulase	Catalase	Oxidase	Pathogen
-	-	+	+	-	+	-	<i>K.pneumoniae</i>
+	-	-	-	-	+	-	<i>E. coli</i>
-	+	-	+	-	+	-	<i>Proteus sp.</i>
+	+	+	-	-	-	+	<i>V. cholerae</i>
+	-	+	+	+	+	+	<i>S. aureus</i>

M. Red; methyl Red, V. Proskauer; Voges Proskauer, C. utilization: citrate utilization, *K.pneumoniae*; *Klebsiella pneumoniae*, *E. coli*; *Escherichia coli*, *V. cholera*; *Vibrio cholerae*, *S.aureus*; *Staphylococcus aureus*.

Table 2. Biochemical tests of the isolates

Pathogen	Zone of inhibition (mm)			
	EU 30	EU 37	EU 41	EU 154
<i>K.pneumoniae</i>	23±0.3	22±0.1	21±0.3	21±0.2
<i>E. coli</i>	22±0.2	18±0.3	23±0.2	20±0.3
<i>Proteus sp.</i>	23±0.2	20±0.2	20±0.2	19±0.3
<i>V. cholera</i>	21±0.3	22±0.3	25±0.3	18±0.2
<i>S. aureus</i>	25±0.2	25±0.1	27±0.2	22±0.3

K. pneumoniae; *Klebsiella pneumoniae*, *E. coli*; *Escherichia coli*, *V. cholera*; *Vibrio cholerae*, *S.aureus*; *Staphylococcus aureus*.

Table 3. Zones of inhibition of the isolated bacteria by metabolites from the selected Actinomyces.

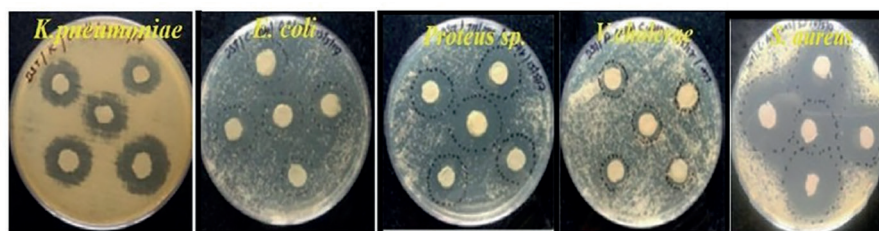


Figure 1. Zones of inhibition of the isolated bacteria by metabolites from the selected Actinomyces.

Pathogen	Minimum inhibitory concentration (mg/ml)			
	EU 30	EU 37	EU 41	EU 154
<i>K.pneumoniae</i>	0.25±0.01	0.24±0.02	0.23±0.03	0.24±0.02
<i>E. coli</i>	0.24±0.02	0.19±0.01	0.23±0.01	0.22±0.03
<i>Proteus sp.</i>	0.25±0.02	0.22±0.02	0.18±0.01	0.18±0.01
<i>V. cholera</i>	0.23±0.03	0.24±0.02	0.25±0.02	0.17±0.02
<i>S. aureus</i>	0.25±0.01	0.23±0.03	0.23±0.01	0.23±0.02

K. pneumoniae; *Klebsiella pneumoniae*, *E. coli*; *Escherichia coli*, *V. cholera*; *Vibrio cholerae*, *S.aureus*; *Staphylococcus aureus*.

Table 4. Minimum inhibitory concentration of the isolated bacteria by metabolites from the selected Actinomycetes.

Discussion

Shallow wells A and C had no viable cells (Table 1). However, viable cells were isolated from wells B, D and E. The lack of viable cells from wells A and C could be attributed to lack of seepage of wastewater in the area 18. The isolation of viable cells from wells B, D and E concurred with a study carried out in Pakistan 23. The similarity in results between the two studies may be attributed to the proximity of the wells to pit latrines 24.

The results on biochemical characterization of the isolates obtained in this study are typical of the isolates (Table 2). According to Joao and Cabra (2014) 25, biochemical characteristics of bacterial isolates are an indispensable tool in their identification. The results of the current study concur with those of a previous study by Poornima (2018) 26. The possible reason for the similarity in the results could be the isolation of the same bacteria 27. Florica *et al.* (2015) asserted that the same strains of bacteria react the same way biochemically.

The zones of inhibition of the bacterial pathogens by the Actinomycetes crude extracts are presented in Table 3. These results differed with a previous study obtained by Waitthaka *et al.* (2017b) 15. Sukhvir *et al.* (2016) 28 explained that the type of antibiotics produced by Actinomycetes determines the zones of inhibition shown by the tested pathogens. Besides, the evolutionary characteristics of the test pathogens also determine the size of inhibition 29.

However, the minimum inhibitory concentrations presented by the metabolites in the current study partially agreed with a previous study by Rajeswari *et al.* (2015) 30. This may be attributed to the Actinomycetes utilizing the same mechanisms of inhibiting the growth of the test pathogens 31. In addition, the concentration of the antibiotics may have led to the observed results 32.

Conclusions

Water from some shallow wells in Dundori is highly contaminated with *K. pneumoniae*, *E. coli*, *Proteus* sp., *V. cholerae* and *S. aureus*. The pathogens can effectively be controlled using antibiotics from the Actinomycetes.

Recommendations

There is a need to sensitize the residents of the study area on ways of preventing seepage of contaminants into the shallow wells. Further studies aimed at determining the me-

chanisms of antimicrobial control of actinomycete need to be carried out.

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Conflict of interest

No conflict of interest declared.

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