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Antimicrobial Activity of 1,3 Dihydroxy-2-Methylimidazolium Bis (Trifluoromethylsulfonyl) Imde and Selected Antibiotics Against *Streptococcus* Species Isolated from Groundwater in the Northwest Province, South Africa

Collins Njie Ateba[1,*], Paul Katlego Morare[1], Eno Ebenso[2]

[1]Department of Biological Sciences, School of Environmental and Health Sciences, Faculty of Agriculture, Science and Technology, North West University- Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa
[2]Department of Chemistry, School of Mathematical and Physical Sciences, Faculty of Agriculture, Science and Technology, North West University – Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa
*Corresponding author: atebacollins1@hotmail.com

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Abstract In developing countries, access to unsafe water and inadequate sanitation continue to be a danger to human health and untreated groundwater constitutes one of the main sources of drinking water supply. The consumption of untreated contaminated groundwater has frequently been identified as a potential source for the transfer and spread of both antibiotic resistant bacteria strains and waterborne infections among humans. Antibiotics are used for the treatment of bacterial infections and therefore the emergence of drug resistant bacterial strains has huge clinical implications. Faced with the rapid growth of bacterial resistance to a number of commonly used antibiotics, ionic liquids are attracting increasing attention as agents that could serve as antimicrobial agents. The aim of this study was to isolate, identify and determine the antimicrobial activity of an ionic liquid and selected antibiotics against *Streptococcus* species isolated from groundwater samples. A total of 22 samples were collected from borehole taps in some villages in the Northwest Province and analysed for the presence of streptococci. Gram-staining, catalase test, oxidase test and Prolex Streptococcal Grouping Latex assays were used for bacteria identification. The growth inhibitory effect of the ionic liquid 1,3 dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imide and selected antibiotics was determined. One hundred and seventy six presumptive isolates were obtained and screened for characters of streptococci and 165 isolates were obtained. All the isolates were Gram positive cocci; a large proportion (92.7%) was oxidase negative and only 51.5% were catalase negative. Based on serotyping, 86.7% of the isolates were positively identified as streptococci. All the isolates from Motlhabeng, Stella and Taung were resistant to penicillin G. Moreover, large proportions (75.0% to 95.8%) of these isolates were resistant to vancomycin. All the isolates except a large proportion of those from Stella (88%) and Taung (83%) were found to be more susceptible (22.5% to 50%) to chloramphenicol. Generally, the growth inhibition zone diameter for 1,3 dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imide against the isolates ranged from 6 to 9 mm and 9 to 22mm for 1% and 10% solutions respectively. In conclusion multi-drug resistant streptococci were isolated from groundwater intended for human consumption. Isolates were however susceptible to the IL tested and the antimicrobial effect increased with increase in concentration of the IL.

Keywords: *Streptococci, ionic liquids, 1,3 dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imide, ionic liquids (ILs), antibiotics, multiple antibiotic resistance, multiple antibiotic resistance phenotypes*

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1. Introduction

Water is a vital natural resource because it is fundamental to life and the environment and is used in food production, industry and power generation [1]. Humans use water for drinking, cooking, recreation, transportation and irrigation [2]. Therefore, the quality of life depends largely on the physical, chemical and microbiological properties of the water to be utilized. Since contaminated water jeopardizes both the physical and social health to consumers, access to portable drinking water is a fundamental human need and therefore, a basic right of every individual [3]. Shortages of water, human activities and industrialization have been found to significantly affect the quality of water globally [4].

In developing countries untreated water and inadequate sanitation pose severe risks to human health [5]. Groundwater constitutes one of the main sources of drinking water supply in rural communities in most African countries [6]. These water sources are usually unprotected and this increases the opportunities for contamination with microbes of faecal origin such as *Escherichia coli, Enterococcus* species and *Salmonella* species [7]. Despite this, in South Africa, residents of most rural communities use untreated groundwater for drinking and household activities [8].

The consumption of untreated contaminated groundwater could serve as a potential source for the transfer and spread of water-borne infections and antibiotic resistant strains [9]. Consequently, the presence of antibiotic resistant isolates in groundwater in rural communities may have severe public health concerns on consumers. In addition, the consumption of contaminated groundwater has been reported to cause a large proportion of outbreaks of waterborne diseases worldwide [7,8,10]. These diseases pose a severe challenge to the medical profession if caused by multiple antibiotic resistant strains [7]. It is therefore beneficial for portable water to be free of microbial contamination thus, reducing the possibility of transmitting disease-causing microbes to consumers [3].

Streptococci and particularly *Streptococcus pneumonia* are a major cause of illnesses that include meningitis, bacteremia, community-acquired pneumonia and acute otitis in their hosts [11]. The treatment of these infections is usually achieved through the administration of antibiotics and therefore the emergence of drug resistant strains often complicates the management of these infections [12]. Antibiotic resistance is a situation in which some sub-populations of microorganisms, usually bacterial species are able to survive after exposure to one or more antibiotics. Resistance to three or more antibiosis is commonly termed multidrug resistance (MDR) [13]. Antibiotic resistance evolves naturally via natural selection through random mutation but it could also be engineered by applying an evolutionary stress on a population. Once an antibiotic gene has been generated in bacteria cells the genetic information can later be transferred to recipient cells in a horizontal fashion by plasmid exchange.

Faced with microbial resistance problems there is need to look for alternative agents that could have potential antimicrobial properties. Ionic liquids are basically salts that are in the liquid phase below 200°C [14]. Ionic liquids (ILs) are attracting increasing attention worldwide due to their unique properties, which include negligible vapor pressure, chemical and thermal stability, non-flammability, high ionic conductivity, low melting points, low viscosity, wide electrochemical potential window, solvation ability and antimicrobial activity [15,16,17,18]. ILs have been shown to exhibit antimicrobial activities against cocci, rods and fungi and there is usually a correlation between the structure of the cation and the antimicrobial activity of the solvent [19]. Due to their unique properties ILs are very useful in both the chemical and biological industries and also serve as successful replacements for some organic solvents used in synthetic [20,21] separation and extraction processes [22,23]. Given their environmental advantage and the fact that some ILs have been shown to possess antimicrobial activities [15,16,17] we evaluated the effect of 1,3-dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imide on streptococci isolated from groundwater. 1,3-dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imide is an ionic liquid with a molecular weight of 409.28 and it is used as an N-heterocyclic carbene (NHC) precatalyst. It is a solid at room temperature and melts at 72°C [24]. The aim of this study was to isolate, identify and determine the toxicity of an ionic liquid and some selected antibiotics against *Streptococcus* species isolated from groundwater collected in some communities in the North West Province, South Africa.

2. Materials and Methods

2.1. Sampling Site and Sample Collection

A total of 22 samples were collected from borehole taps in some rural communities in the North West Province. These included 5 samples each from Ramosadi, Dibate and Motlhabeng and 1 from Stella, and 3 each from Vryburg and Taung. Water samples were collected aseptically, transported on ice to the laboratory for analysis.

2.2. Analysis of Water Samples

Water samples were analysed immediately upon arrival in the laboratory. Analysis was performed according to standard methods [25]. An aliquot of 100 mL from each sample was filtered using 0.45 µm filter paper (Watman Glass Microfiber GS Filter paper) on a water pump machine (model Sartorius 16824). Membrane filters were transferred onto Brain Heart Infusion (BHI) agar plates and plates were incubated aerobically at 37°C for 24 to 48 hours. Typical white centred colonies were sub-cultured on BHI agar plates and plates incubated aerobically at 37°C for 24 to 48 hours. Eight presumptive *Streptococcus* isolates from each sample were randomly selected and their identities confirmed using primary and secondary identification tests.

2.3. Bacterial Identification

Presumptive isolates were identified using the following criteria:

2.3.1. Cellular Morphology

Presumptive isolates were Gram stained using standard methods [26].

2.3.2. Preliminary Biochemical Identification Tests for Streptococci Species

2.3.2.1 Oxidase Test

The oxidase test was performed using the oxidase test reagent from Pro-Lab Diagnostics- United Kingdom and performed as instructed by the manufacturer (Whatman International Ltd, Maidstone, England).

2.3.2.2 Catalase Test

The catalase test which is designed to detect the presence of the catalase enzymes in most aerobic and facultative anaerobic bacteria based on the presence of the cytochrome system was determined in all the isolates. Isolates from Gram positive and coccid shaped cultures were reacted with 2% (v/v) hydrogen peroxide on a clean microscope slide. The formation of bubbles was considered a positive result and vice versa.

2.3.3. Confirmatory Biochemical Test for Streptococci

2.3.3.1 Serotyping

A serological Prolix Streptococcal Grouping Latex agglutination kit obtained from PRO-LAB Diagnostics-UK was used to identify the isolates as streptococci based on the Lancefield grouping. The tests were performed by reacting cell wall specific carbohydrate extract of isolates with nitrous acid reagents as indicated by the manufacturer. A *Streptococcus agalactiae* 12386™ (ATCC 301754) and *Staphylococcus* strain (ATCC® 43300) were reacted with the streptococcal positive control latex reagent provided by the manufacturer for quality control purposes.

2.4. Antibiotic Susceptibility Testing

Antibiotic susceptibility tests were performed on all *Streptococcus* isolates to determine their antibiotic resistant profiles using the Kirby-Bauer disc diffusion technique [27]. Before antibiotic sensitivity testing, the isolates were sub-culturing onto the BHI agar plates and incubated aerobically at 37°C for 24 hours. Bacterial suspensions of pure isolates were prepared and 100 µL aliquots from these suspensions were spread-plated onto Mueller Hinton agar (Biolab, Merck, South Africa). The susceptibilities of the isolates against a panel of six different antibiotics obtained from Mast Diagnostics-United Kingdom were determined. The antibiotic discs were gently pressed onto the inoculated Mueller Hinton agar to ensure intimate contact with the surface and plates were incubated aerobically at 37°C for 24 hours [28]. The antibiotic inhibition zone diameters were measured and results obtained were used to classify isolates as being resistant, intermediate resistant or susceptible to a particular antibiotic based on standard reference values [28]. Table 1 indicates the details of antibiotics used in the study.

Table 1. Details of antibiotics used in the study

Antibiotic	Disc conc.	R	I	S
Ampicillin (AP)	10µg	≤11	12-14	≥15
Penicillin (PG)	10µg	≤20	21-28	≥29
Vancomycin (VA)	30µg	≤9	10-11	≥12
Tetracycline(T)	30µg	≤14	15-18	≥19
Chloramphenicol(C)	30µg	≤12	13-17	≥18
Erythromycin(E)	15µg	≤13	14-22	≥23

2.5. Anti-Icrobial Activity of 1,3 Dihydroxy-2-Methylimidazolium Bis (Trifluoromethylsulfonyl) Imide

The toxicity of the 1,3 dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imide on confirmed *Streptococcus* isolates was performed as previously described [29]. Solidified Muller Hinton agar was spread-plated with a dense suspension of the bacteria isolates. The inoculated agar was supplied with 6 mm diameter sterile paper discs that were soaked in two different concentrations (1% and 10%) (v/v) of the ionic liquid. Plates were incubated at 37°C for 24 hours and the diameters of the zones of growth inhibition around the discs were measured and reported in mm.

3. Results and Interpretation

3.1. Detection of Streptococci Using Biochemical Tests and Serological Assays

A total of 22 groundwater samples were collected from borehole taps and analyzed for characters of streptococci. The number of isolates that satisfied both the preliminary (Gram staining, oxidase test and catalase test) and confirmatory identification test (serotyping) are shown in Table 2. A total of 165 presumptive isolates were screened for characters of streptococci and all the isolates were Gram positive cocci. When subjected to the oxidase test, large proportions (92.7%) of these isolates were oxidase negative while 51.5% were catalase negative. Based on serological assay, 86.7% of the isolates were positively identified as *Streptococcus* species.

Table 2. Preliminary test results for the isolates obtained during the study

Sample station	GS (+ve coccus)	OT (-ve)	CT (-ve)	Serotyping (No. of isolates positively identified as streptococci)
Ramosadi (NT=40)	40	37	0	34
Dibate (NT=40)	40	31	0	38
Motlhabeng (NT=29)	29	29	29	29
Stella (NT=8)	8	8	8	5
Vryburg (NT=24)	24	24	24	20
Taung (NT=24)	24	24	24	17
Total	165	153	85	143

NT =Number tested; GS=Gram staining; OT=Oxidase test; CT= (Catalase test;

3.2. Percentage Antibiotic Resistance of *Streptococci* Species

All the *Streptococci* isolated were tested to determine their antibiotic susceptibility profiles to a panel of 6 different microbial agents and results obtained are shown in Table 3. All the isolates from Motlhabeng, Stella and Taung were resistant to penicillin G. Moreover, large proportions (75.0% to 95.8%) of these isolates were also resistant to vancomycin. In addition, large proportions (75% to 100%) of the isolates from Taung, Motlhabeng, Dibate and Ramosadi were resistant to erythromycin (Table 3).

3.3. Toxic Effects of 1,3 Dihydroxy-2-Methylimidazolium Bis (Trifluoromethylsulfonyl) Imide against *Streptococcus* Species

The growth inhibitory effect of the ionic liquid 1,3 dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imide was determined. Generally, the growth inhibition zone diameter for the isolates ranged from 6 to 9 mm and 9 to 22 mm for 1% (v/v) and 10% (v/v) solutions respectively of the ionic liquid.

Table 3. Antibiotic resistance data for streptococci obtained during the study

Sample source		AP	PG	VA	T	C	E
Ramosadi (NT=40)	NR	28	40	40	22	9	40
%	%	70	100	100	55	22.5	100
Dibate (NT=40)	NR	31	40	0	18	20	39
%	%	77.5	100	0	45	50	97.5
Motlhabeng (NT=29)	NR	21	28	21	22	8	25
%	%	75	100	75	78.6	28.6	89.3
Stella (NT=8)	NR	7	8	6	8	7	1
%	%	88	100	75	100	88	13
Vryburg (NT=24)	NR	15	16	10	16	9	13
%	%	63	67	41.7	67	38	54
Taung (NT=24)	NR	24	24	23	24	20	18
%	%	100	100	95.8	100	83	75

NT= Number tested, NR = Number resistant, % = Percentage resistant,, VA =Vancomycin, PG = Penicillin, AP = Ampicillin, E =Erythromycin, C = Chloramphenicol, T =Tetracycline

4. Discussions

Underground water is an aquatic biotope that harbours different types of micro-organisms [6]. The evolution and composition of the bacterial micro-flora depends on several factors such as the bacteria and solid particles mobility, the hydrodynamics and the hydrochemistry of the groundwater among others [10,30]. Groundwater constitutes one of the main sources of drinking water supply in most African cities [6]. The bacteriological quality of drinking water is responsible in the short-term for the immediate health risks on consumers and is therefore the most estimated property [31]. The distribution of bacterial species in aquatic environments in general and underground water in particular, is greatly influenced by environmental factors. Despite the fact that there is currently no data is available on the role of environmental factors on the bacterial community in groundwater in the study area, some studies have indicated the presence of microbial pathogens of faecal origin in groundwater intended for human consumption [7,8]. The present study was aimed at analyzing groundwater from some rural communities in the North West Province for the presence of faecal streptococci, a bacterial group of high medical importance especially in young children, elder and immune-compromised individuals.

Life depends on water and access to safe drinking water is essential [32]. In addition, access to portable drinking water is a fundamental human need and therefore a basic right of every individual, since contaminated water jeopardizes both the physical and social health to consumers [3]. In the present study, streptococci were successfully isolated from groundwater consumed by individuals in some rural communities in the North West Province, South Africa. The identities of these isolates were confirmed using Gram staining, oxidase test, catalase test and a serological assay. These same methods have been used for identification of *Streptococcus species* [33,34,35]. However, these tests are supplemented with molecular assays the isolates are identified at species level [35].

Another objective of this study was to determine the antibiotic resistance profiles of isolates obtained from water samples. The antibiotic resistance patterns of *Streptococcus* species from food products and humans have been investigated [36,37,38,39]. In the present study large proportions (75.0% to 100%) of the isolates were most often resistant to penicillin G, vancomycin, and erythromycin. In the present study, isolates from different sample sites differed in their antimicrobial resistance patterns and similar observations have been reported [40]. In some of these studies resistance to tetracycline was the most common, followed by resistance to erythromycin, pirlimycin, and gentamicin [39]. Moreover, *Streptococcus* species have been reported to marked susceptibility to β-lactam antibiotics [37] and this is contrary to our finding. *S. iniae* isolates obtained from olive flounder (*Paralichthys olivaceus*) displayed susceptibility to cefotaxime, erythromycin, ofloxacin, penicillin, tetracycline and vancomycin when compared *S. parauberis* isolates that were highly resistant to erythromycin and tetracycline [38]. It is therefore suggested that the antibiotic resistance patterns of isolates within the same genus may differ despite the fact that they occupy the same ecological niche. This amplifies the need for constant testing and monitoring.

Antimicrobial resistance seems to be influenced by a number of factors that are unique to different areas and multi-drug resistance has become a primary issue of concern to physicians since it greatly affects therapeutic processes [41]. Therefore, as multi-drug resistant strains become increasingly prevalent, treatment options become limited [41]. Mortality associated with pneumococcal infections dropped dramatically following the advent of penicillin therapy [42]. However, drug-resistant strains, including those with reduced susceptibility to multiple antibiotics became increasingly prevalent in many parts of the world later on. The emergence of resistant strains greatly affected the options of antibiotics that may provide reliable and effective treatment of pneumococcal infections [11]. In the present study, all the isolates were found to be resistant to ampicillin (63% to 100%) and this may have resulted from the fact that the drug is frequently used in both veterinary and human medicine in the area. In addition isolates except those from Stella (88%) and Taung (83%) were highly susceptible to chloramphenicol. This may have resulted from the fact that chloramphenicol has been banned for inclusion as additives in feeds for animals [43]. Moreover, it is not used in veterinary medicine in the study area.

A further objective was to determine the effect of an ionic liquid, 1,3-dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imide against streptococci isolated from groundwater samples. Generally, the ionic liquid had visible antimicrobial activity against the isolates and the degree of susceptibility depended greatly on the concentration of the liquid utilized. In the present study growth inhibition halo ranged from 6 to 9 mm and 9 to 22mm for 1% and 10% solutions respectively. Similar to a previous report, the ability to induce apoptosis in Streptococcus cells dependent on the concentration of dispersions [44]. Room temperature ionic liquids have shown interesting chemical and biological properties and are effective against certain Gram-negative as well as Gram-positive bacteria [45]. They achieve these antimicrobial properties by affecting some proteins such as bovine serum albumin (BSA) and catalase (CAT) that are central to the survival of the cell [45]. In addition, several ionic liquids have been screened for antimicrobial activity activities, and results obtained were both interesting and promising [46]. To the best of our knowledge no study has been conducted in which 1,3 dihydroxy-2-methylimidazolium bis (trifluoromethylsulfonyl) imde was assayed to determine its antimicrobial activities. Despite this, two similar long-chain imidazolium and pyridinium based ionic liquids namely 1-alkyl-3-methylimidazolium and 1-alkylpyridinium bromides have been assessed against microorganisms to determine the contribution of the alkyl chain length and the nature of the cationic head group on the antimicrobial properties of the ionic liquids [47]. In the present study the imidazolium-based ionic liquid had negative effects on the growth of *Streptococcus* species isolated and similar observations have been reported for other imidazolium compounds [48]. However, in the previous study the inhibitory activity was highly dependent the type of anion present and the length of the alkyl chain.

5. Conclusion

In conclusion, the occurrence of multi-drug resistant isolates in developing countries is a course for concern as infections caused by these bacteria could have severe consequences on individuals. Their effects could even be amplified in rural communities where residents do not practice proper hygiene and have limited access to health care facilities. Proper water management practices and efforts to provide portable water to residents in rural communities cannot be overemphasized. This will limit the health implications on humans that result from consuming untreated ground water.

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