Microbes and Infectious Diseases 2022; 3(2): 472-483

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Upshot of virulence markers and effects of temperature and pH on haemolytic bacteria in South-West Nigeria

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ARTICLE INFO

Article history:
Received 22 January 2021
Received in revised form 10 March 2021
Accepted 22 March 2021

Keywords:
ß-haemolytic bacteria
Capsule
pH
Stream
Temperature

ABSTRACT

Background: Virulence is the extent of pathogenicity displayed by majority of pathogens and yardstick that efficiently distinguishes pathogenic and non-pathogenic organisms. Effects of pH, temperature and incubation period were studied on capsule-positive bacteria isolated from Onyearugbulem stream, Akure. **Methods:** Water samples from Onyearugbulem stream in Akure was collected in sterile 500ml sample bottles. Water samples obtained from the stream were subjected to microbiological analysis. Implicated bacteria were tested for haemolysis and virulence determination via blood agar (5% v/v) and for the presence of capsule using India ink. ß-haemolytic bacteria were subjected to different temperature (15 °C – 60 °C) and pH (6.0 – 9.0) ranges. pH conditions were achieved by the addition of 0.01M NaOH and 0.01M HCl to media before sterilization. The zones of clearance (mm) were measured at 24 h, 48 h and 72 h. **Results:** *Proteus penneri* showed the highest haemolytic activity (56 mm) at 28 °C after a duration of 72 h. *Bacillus cereus* showed the highest haemolytic activity (52 mm) at pH 8.5, after 72 h Dye degradation was optimum at 10 to 12 h at 37 °C which showed the haemolytic bacterial organisms were capsule-positive. **Conclusion:** The findings in this study revealed that bacteria present in Onyearugbulem stream contained virulent factors with highest activity at ambient temperature (28 °C) which indicate the poor quality of the stream and thereby constitute serious health threat to man and animals.

Introduction

Water is one of the most important and most precious natural resources. It is essential in the life of all living organisms from the simplest plant and microorganisms to the most complex living system known as human body [1]. Water needs have had serious socio-economic and health influences on urban development in developing countries where population concentration has put serious strains on available resources [2]. Human interaction can jeopardize parts of this system in a variety of ways. One principal way is through the runoff of fertilizers or sewage into a water body. Both contain nutrients that plants, algae, and cyanobacteria can use to grow and excessive nutrient amounts can lead to very rapid growth [3]. Several pollutants such as chemicals, sewage and household waste can be discovered in surface water bodies such as lakes, streams etc, but it could be said that microorganisms are the most important pollutant on surface water because they are living organism with the ability to thrive on other forms of pollutants and surface waters provide an environment for the growth and replication of a wide range of microorganisms.

Microbial pollution in aquatic environments is one of the crucial issues with regard

DOI: 10.21608/MID.2021.64549.1128
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to the sanitary state of water bodies used for drinking water supply, recreational activities and harvesting seafood due to a potential contamination by pathogenic bacteria, protozoa or viruses [4]. Most waterborne pathogens are introduced into drinking-water supplies in human or animal faeces [5] and *Escherichia coli* is found in high concentration in all mammalian faeces [6], microorganisms can also be introduced into surface water through natural occurrences such as rainfall, the occurrence of erosion also contributes to the introduction of microorganisms into surface water. The kind of microorganisms which can be found in surface water have a wide variety and this is because water provides an environment that is suitable for the growth of several microorganisms excluding the ones which live in the extreme conditions like the extremophiles, hyperthermophiles and also provides the necessary nutrients for microbial growth that would certainly hinder the survival of almost all eukaryotic organisms [7]. River water is usually contaminated by bacteria (e.g., *Escherichia coli*, *Clostridium perfringens*), viruses (e.g., adenovirus) and pathogenic protozoa (e.g., *G. duodenalis*, *C. parvum*) [6].

Microorganisms are greatly affected by the chemical and physical state of their environment, and four factors control growth in a major way: temperature, pH, water availability, and oxygen [3]. Other environmental conditions which influence the growth of microorganisms include radiation and pressure. Such environmental conditions for growth are also referred to as physicochemical parameters for growth. Each of the major and minor physicochemical parameters for microbial growth plays major roles in the proper function of microorganisms e.g. oxygen (O_2) is an essential nutrient; they are unable to metabolize or grow without it. Other organisms, by contrast, cannot grow in the presence of O_2 and may even be killed by it [3] and radiation (sunlight) determines if microorganisms carry out certain chemical reactions. The pH of surface water goes a long way in determining the kind of microorganisms that would thrive in them as each class of microorganisms have pH range in which they thrive. Different microbial groups have characteristic pH preferences.

Most bacteria and protozoa are neutrophiles. Most fungi prefer slightly acid surroundings, about pH 4 to 6; algae also seem to favour slight acidity [8]. **Lansing et al.** [3] also stated that they may be exceptions to the groupings of microorganisms based on their pH giving examples such as the Archaea; *Ferroplasma acidarmanus* and *Picrophilus oshimae* that can actually grow at pH 0. Microorganisms however grow over a wide range of pH in a bid to adapt to their environments. This however suggests that surface water would have higher population of microorganisms that are neutrophilic. Most microorganisms grow well at different temperature ranges. The temperature of an environment is a vital factor for the multiplication of cells. Microorganisms however depend on temperature for survival with each organism having the temperature at which it grows best and may not grow when below or above that temperature range, Although as indicated by **Lansing et al.** [3], several microorganisms learn to adapt to environments which do not fall in their ideal temperature range in order to survive. So every microorganism has a particular temperature at which it grows best and also a minimum growth temperature and maximum growth temperature.

The acidity or alkalinity level (pH) and the temperature of surface water bodies may not have constant values owing to several factors such as human activities, climate change, industrialisation, urbanisation and biological activities of aquatic organisms present in these water bodies. Virulence is a mode of pathogenicity displayed by most pathogens and it is a yardstick that efficiently differentiates pathogenic and non-pathogenic species of bacteria and fungi [9]. The staining method using India ink is an appreciative and cost-effective for initial screening of bacteria as it ascertained the presence and absence of certain virulence-determining characters such as presence of capsules [10]. The proficiency of utilizing India ink depends on various factors such as origin of stain, staining techniques, need for good microscope and visual observation and inference. **Price** [11] and **Avila-campos et al.** [12] has shown that the measure of virulence depends on many virulence determinants like haemolysin which is overly connected to the beta-haemolytic bacteria subjected to virulence test in this study.

Thus, the purpose of this study is to appraise the effects of pH, temperature and incubation period on capsule-positive and ß-haemolytic bacteria isolated from Onyearugbulem stream, Akure, South-West Nigeria.

Materials and Method

Study site

Onyearugbulem stream with coordinates 7.2810 ºN, 5.1905 ºE is one of the tributaries and river catchment of Ala river which is a main river in Akure metropolis. The stream navigates through the densely-populated Onyearugbulem Estate along Akure-Owo Express-way.

Water sampling techniques

Water samples from Onyearugbulem stream in Akure was collected in sterile 500 ml sample bottles very early in the morning and taken to the laboratory for microbiological analysis within 1 hour as indicated by **Makanjuola et al.** [13].

Microbiological analysis

Isolation of tested organisms

Water samples obtained from the stream were subjected to microbiological analysis. One millilitre of each water sample was diluted in 9 ml of sterile distilled water, followed by fourth to ninth fold serial dilutions (10-4 to 10-9). One tenth of a millilitre of the fourth to ninth fold diluents were plated out in triplicates on Eosin Methylene blue agar (Oxoid, UK) and Nutrient agar (Oxoid, UK) media according to **Makanjuola et al.** [13] Distinct colonies are then sub-cultured on nutrient agar plates and identified using molecular identification as described by **Gurakan et al.** [14] and **Sambrook et al.** [15]. Blood agar was then used for the identification of haemolytic organisms.

Mycological analysis of stream water samples through serial dilution as described by **Al-mohanna** [16]. Usually, the recommended diluent was aqueous 0.1% peptone. Serial dilutions (10-4 to 10-9) were carried out and the pour plate method via Potato dextrose agar (PDA) (Oxoid UK). Plates were incubated and enumerated. The fungal isolates were examined macroscopically and microscopically to determine their colonial and morphological characteristics respectively according to **Agbabiaka et al.** [1].

16S reverse ribose nucleic acid gene amplification of bacterial isolates from the water samples

Polymerase chain reaction (PCR)-based naming and categorization of bacterial isolates was carried out via DNA extraction of bacteria isolates according to the technique illustrated by **Gurakan et al.** [14]. PCR expansion was carried as demonstrated by **Sambrook et al.** [15]; the PCR profile used is initial denaturation temperature of 94 ˚C for 3 mins, followed by 30 cycles of 94 ˚C for 60 sec, 56 ˚C for 60 sec, 72 ˚C for 120 seconds and the final extension temperature of 72 ˚C for 5 minutes followed by sequencing and genetic make-up blasting via the National centre for biotechnological information (NCBI) server. PCR-sequencing was conducted via DNA Sanger sequencing and data was analyzed by ABI Sequencing Analysis software (version 5.2).

Haemolytic test activity of isolated bacteria

Haemolysis test was carried out using blood agar, isolated bacteria were introduced onto blood agar by streaking and the reaction of the isolates on the plates were designated as: alpha (α) haemolysis which indicates a greenish cloudy zone around the colony; beta (β) haemolysis: also known as complete lysis, a clear zone with a clean edge around the colony and gamma (γ) haemolysis in which lysis does not occur indicating no change in the blood agar around the colony. Each bacterial strain was tested for its haemolytic activity on different blood agar plates using cow blood as exhibited by **Lippi et al.** [17]. Blood agar plates were incubated at desired temperature (to determine the effect of temperature) for 24 h and at desired pH at 37 °C. The zone of clearance was noted at 24, 48 and 72 h for each bacterium [18].

Increasing and decreasing of pH

A standardized pH meter (model PHS-3C) with which glass electrode was first rinsed with distilled water before being dipped into the prepared media, after which the pH stabilizes, 0.01 molar NaOH was added to prepared media and stirred before checking stabilized pH value until desired pH is reached. This process was carried out before sterilization of the blood agar media.

A standardized pH meter (model PHS-3C) in which glass electrode was first rinsed with distilled water before being dipped into the prepared blood agar media and thereafter the pH reading was stabilized, 0.01 molar HCL was added to prepared blood agar and stirred before checking stabilized pH value until desired pH is reached. This process was carried out before sterilization of the media as demonstrated by **Khan et al.** [19].

Adjustment of temperature

Prepared media was allowed to solidify and microorganism to be tested was introduced into the plate by streaking, plates are then placed in the oven and the oven was regulated to desired temperature

ranges of 15 ºC to 60 ºC. Blood agar was then used for the identification of haemolytic organisms as depicted by **Khan et al.** [19].

Virulence determination of bacteria

Tryptic Soy Broth (TSB) (Hi-media, India) was prepared. India ink (Dye based Ink) (Matador Ltd., Nigeria) was filtered through millipore membrane filter with 0.22 µm pore sizes (Matador, Nigeria) and added to the broth at a sole concentration of 10 %. Selected bacterial isolates were also streaked on nutrient agar plate to a final concentration of 0.01 % in tryptic soy agar (TSA) (Hi-media, India) medium and incubated in anaerobic condition A 24 h bacterial culture at 50 - 60 µl was inoculated to the broth using a volume range micropipette (Matador Ltd., Nigeria) and incubated for 24 h at 37 ºC and checked at definite time interval up to 48 has illustrated by **Behera et al.** [20].

Data analysis

Data were expressed as the mean ± Standard error mean (SEM) of experiments performed in duplicates. One-way analysis of variance (ANOVA) was taken to be significant at $p>0.05$ using GraphPad Prism version 5.0.

Results and discussion

This study was carried out to ascertain the upshot of virulence markers and effects of temperature and pH on haemolytic bacteria isolated from Onyearugbulem stream, Akure. After carrying out the haemolysis test on the isolated bacterial organisms, 4 beta haemolytic bacterial isolates were selected due to their medical importance and the influence of temperature values ranging from 15 °C to 60 °C and pH ranging from 6.0 to 9.0 was examined as each of these bacterial haemolytic activity were shown in its zone of clearance (mm).

Haemolytic activity of isolated bacteria

Proteus mirabilis and *Alcaligenes faecalis* displayed gamma haemolytic activity. *Alcaligenes faecalis, Bacillus cereus, Stenotrophomonas acidiminiphilis* and *Proteus penneri* showed beta haemolytic activity while *Lysinibacillus macrolides* was alpha haemolytic **(Table1)**. Some of these bacteria were also isolated in the study carried out by **Agbabiaka et al.** [1] on Foma River Ilorin, the similar microorganisms were *Serratia spp, Proteus mirabilis, Bacillus cereus* and *Proteus penneri*. The presence of these microorganisms in Onyearugbulem stream could be strongly linked to human-related activities as supported by **Agbabiaka et al.** [1].

Bacterial organisms isolated from the stream water samples

All the bacteria isolated obtained presumptively were biotechnologically-identified including; *Proteus mirabilis* HI4320, *Alcaligenes faecalis* ZD02, *Alcaligenes faecalis* B137W, *Bacillus cereus* ATCC 14579, *Stenotrophomonas acidiminiphilis* AMX19, *Lysinibacillus macroides* ARS5 and *Proteus penneri* NCTC 12737 as illustrated in **table (2)**. This finding is in alliance with the observation of **Agbabiaka et al.** [1] who enumerated *P. mirabilis, B. cereus* and *P. penneri* from Foma River Ilorin.

Presumptive fungal profile of water samples

Aspergillus niger, *Mucor mucedo* and *Aspergillus fumigatus* were fungal organisms implicated in the mycological analysis of stream water samples as juxtaposed in **table (3)**. This is in accordance with **Agbabiaka et al.** [1].

Effects of temperature on beta-haemolytic activity of bacteria

Figure 1-4 shows the influence of temperature on the haemolytic activity of the four beta haemolytic bacterial organisms in this study including *Stenotrophomonas acidaminiphilis, Alcaligenes faecalis, Bacillus cereus* and *Proteus penneri*. Haemolytic activity occurred at different temperatures and it also increases with the duration of incubation time. All the organisms subjected to temperature change had lower beta-haemolytic activity at 37 ºC than 28 ºC, *Bacillus cereus* had the highest haemolytic activity at 37 ºC but at 28 ºC it had the lowest of haemolytic activity. *Proteus penneri* and *Alcaligenes faecalis* showed growth at 55 ºC but growth did not occur at 60 ºC.

Haemolysis occurred at 28 ºC and 37 ºC for all of the organisms but was higher at 28 ºC. This study revealed that the beta-haemolytic bacteria selected due to their relatively high pathogenicity bears comparism to other kinds of bacteria. This statement was acknowledged by **Bodade et al.** [18] and **Kumar et al.** [21] in their study on haemolytic bacteria. Selected beta-haemolytic bacteria subjected to different temperature values 15 ºC, 28 ºC, 37 ºC, 55 ºC, and 60 ºC, expectedly showed different levels of zone of clearance for haemolysis. Beta-haemolysis occurred for all bacteria subjected to haemolysis at 28 ºC and 37 ºC with the highest zone of clearance exhibited at 28 ºC for all of the isolates, in line with **Bodade et al.** [18] in his study on beta-haemolysis which had more haemolysin released at 37 than at 30 ºC indicating that

haemolysis occurred more at 37 ºC, this variability could be attributed to the inability to maintain stable temperature ranges. It was also observed for most of the organisms that haemolytic activity increased with the duration of incubation, the zone of clearance increased by the day, at 24 h all the organisms tested had beta-haemolytic activities between 2 mm - 4 mm and at 48 h the beta-haemolytic activity had doubled and triple in some cases such as *Proteus penneri* and *Alcaligenes faecalis*. The red blood cells (on the plate) were all lysed at 72 h of incubation and these could be attributed to the fact that the substances responsible for haemolysis are enzymes (metabolites) thus agreeing with **Bodade et al.** [18] and **Kumar et al.** [21] so the level of the production of haemolysin increases with the phase of growth. Haemolysis did not occur at 15 ºC, 55 ºC and 60 ºC temperature ranges which do not provide favourable conditions for the growth of some of the haemolytic organisms which in turn does not favour haemolysis. However in contrast to **Bodade et al.** [18], species of *Streptococcus*, *Shigella* and *Staphylococcus* produced haemolysin at a temperature of 50 ºC on sheep and cow blood agar. This study further revealed bacteria such as *Proteus penneri* and *Alcaligenes faecalis* showed growth at 55 ºC but growth did not occur at 60 ºC, indicating that these organisms are thermophilic.

Haemolysis occurred at 28 ºC and 37 ºC for all of the organisms but was higher at 28 ºC, the beta-haemolytic activity at 37 ºC is however of concern as it could be considered as the pseudo-environment to ascertain what could take place if microorganism gain access into the human system. All the organisms subjected to temperature change had lower beta-haemolytic activity at 37 ºC than 28 ºC, *Bacillus cereus* had the highest haemolytic activity at 37 ºC but at 28 ºC it had the lowest rate of haemolytic activity, since *Bacillus cereus* has the highest rate of beta-haemolytic activity it would be of highest importance followed by *Alcaligenes faecalis*, *Stenotrophomonas acidaminiphilis* and *Proteus penneri* at 37 ºC.

Figure 5-8 shows the influence of pH on the haemolytic activity of four the four beta-haemolytic organisms in this study including *Stenotrophomonas acidaminiphilis*, *Alcaligenes faecalis*, *Bacillus cereus* and *Proteus penneri*.

Haemolysis occurred at all of the pH conditions, the organisms however displayed different beta-haemolytic activity at different pH values which also increases with incubation time. At the pH of 6.5, all the test organisms displayed beta-haemolysis except *Alcaligenes faecalis* which only grew at pH 6.5 and since *Stenotrophomonas acidiminiphilis*, showed beta-haemolytic activity at 6.0 and 6.5 and had the highest rate of haemolytic activity at 6.5. At pH 7.0, haemolysis occurred in cultured plates with *Stenotrophomonas acidiminiphilis* having the highest rate of beta-haemolytic activity, *Alcaligenes faecalis* only showed growth at 24 h of incubation and beta-haemolysis occurred at 48 h. At pH 8.5 and 9.0 which is more alkaline, haemolysis occurred in the entire test organisms except *Alcaligenes faecalis*. Haemolysis occurred at all of the pH conditions, the organisms however displayed different beta-haemolytic activity at different pH values and just like the temperatures ranges, the haemolytic activity increased with incubation time, the lowest rate of beta-haemolytic activity was recorded at pH 6.0 at 24 h of incubation growth which did not occur for any of the beta- haemolytic organisms except *Proteus penneri* at 48 h of incubation growth occurring for all of the organisms except *Alcaligenes faecalis* which eventually did not grow in these conditions as its best medium of growth is an alkaline environment. Beta-haemolysis occurred at 48 h of incubation of *Stenotrophomonas acidaminiphilis* and at 72 h the beta-haemolytic activity was the same as 48 h, this however strengthens the fact that haemolysis takes place only under conditions favourable for the optimum growth of test organism as it has been established that the rate of enzymes released increases with the incubation time. At the pH of 6.5, all the test organisms underwent beta-haemolysis except *Alcaligenes faecalis* which only grew at pH 6.5 and since *Stenotrophomonas acidaminiphilis*, showed beta-haemolytic activity at 6.0 it also showed beta-haemolytic activity at 6.5 and had the highest rate of haemolytic activity at 6.5 then *Bacillus cereus* strain and *Proteus penneri* also showed beta-haemolytic activity and these activity increased with incubation but the increase in haemolytic activity was lesser in comparison with when the test organisms were subjected to varying temperature. At pH 7.0, haemolysis occurred in all of the cultured plates with *Stenotrophomonas acidaminiphilis* having the highest rate of beta-haemolytic activity, *Alcaligenes faecalis* however only showed growth at 24 h of incubation and beta-haemolysis occurred at 48 h suggesting that *Alcaligenes faecalis* at these growth condition produces the enzyme responsible for beta-

haemolysis which bears similarity with **Alexander et al.** [22] as they emphasized the significance of temperature and pH on peak enzyme production in bacteria. At pH 8.5 and 9.0 which is more on the alkaline side of the scale haemolysis occurred in all of the test organisms except *Alcaligenes faecalis*, these test organisms however grew in the alkaline medium but did not show signs of haemolysis. *Alcaligenes faecalis* had the highest rate of beta-haemolytic activity in alkaline growth condition where all the red blood cells in the petri dish were lysed and for all of the test organisms, the rate of beta-haemolysis doubled at 48 h of incubation. At 24 h of incubation at a pH condition of 9.0 *Bacillus cereus* and *Proteus penneri* showed signs of haemolytic activity barely at 1 mm but the haemolytic activity increased drastically at 48 h and 72 h of incubation *Bacillus cereus* had lysed almost all the blood cells in the plate. When subjected to different pH conditions Bacillus cereus was found to thrive better at pH 8.5 and *Stenotrophomonas acidiminiphilis* at pH 7.0.

Virulence profile of isolated bacteria

India ink was used for virulence test determination of isolated bacterial and fungal organisms. Dye degradation was optimum at 10 to 12 h at 37 °C for bacteria which showed the haemolytic bacterial organisms were capsule-positive. Growth of isolated bacterial organisms in India ink including culture broth revealed some isolates started degrading the ink after duration of 5 to 6 h in 10% India ink. This is in correlation with the work of **Behera et al.** [20]. This goes further to show that by keeping the culture in 10% India ink, the dye degradation was optimum at 10 to 12 h at 37 °C for bacteria which also bears similarity to **Behera et al**. [20]. This likely confirms that the isolated bacteria have a propensity to be virulent. By increasing the duration of culture time for more than 24 h up to 48 h, all the bacteria isolates degraded the colour in 10% India ink, which is an observation supported by **Behera et al.** [20].

Table 1. Gram reaction of bacterial isolates and their haemolytic activity.

Bacterial Isolate	Shape	Haemolysis test
Isolate 1	Gram +ve rod	Gamma haemolysis
Isolate 2	Gram +ve rod	Beta haemolysis
Isolate 3	Gram –ve rod	Gamma haemolysis
Isolate 4	Gram +ve rod	Beta haemolysis
Isolate 5	Gram –ve rod	Beta haemolysis
Isolate 6	Gram +ve rod	Alpha haemolysis
Isolate 7	Gram –ve rod	Beta haemolysis

Table 2. Molecular identification of multidrug resistant bacterial isolates.

Isolate codes	Biochemical identity	Molecular identity	Percentage similarity	Accession number
Is 1	*Proteus mirabilis*	*Proteus mirabilis*	85 %	HI4320
Is 2	*Alcaligenes faecalis*	*Alcaligenes faecalis*	85 %	ZD02
Is 3	*Alcaligenes faecalis faecalis*	*Alcaligenes faecalis faecalis*	85 %	B137W
Is 4	*Bacillus cereus*	*Bacillus cereus*	93 %	ATCC 14579
Is 5	*Stenotrophomonas acidiminiphilis*	*Stenotrophomonas acidiminiphilis*	93 %	AMX19
Is 6	*Lysinibacillus macroides*	*Lysinibacillus macroides*	97 %	ARS5
Is 7	*Proteus penneri*	*Proteus penneri*	93 %	NCTC 12737

Keys: Is 1- Isolate 1; Is 2- Isolate 2; Is 3- Isolate 3; Is 4- Isolate 4; Is 5- Isolate 5; Is 6- Isolate 6; Is 7- Isolate 7

Table 3. Morphological description of fungal isolates.

Colony description	Morphological characteristics	Suspected fungal organism
Black colonies	Septate branched mycelium, brownish conidia, ascopores produced.	*Aspergillus niger*
Grayish brown colonies	Broad hyphae, non-septate Sporangiophores	*Mucor mucedo*
White, yellow- brown colonies	Ascospores produced, short conidia, septate and broad hyphae	*Aspergillus fumigatus*

Figure 1. Effect of temperature on the haemolytic activity of *Stenotrophomonas acidaminiphilis*

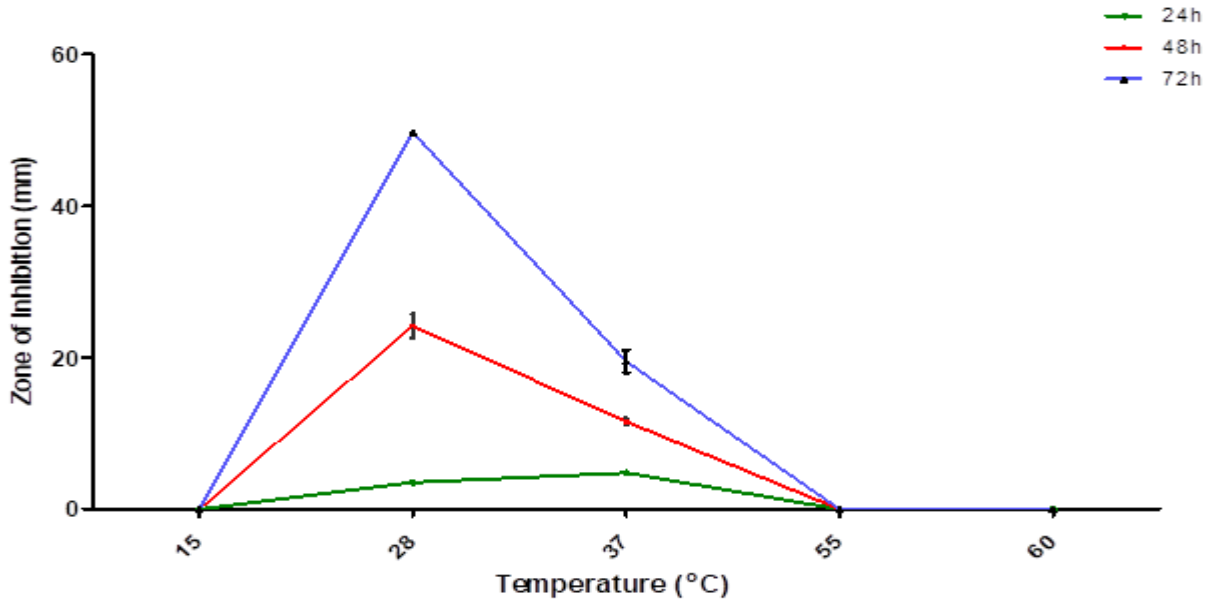


Figure 2. Effect of temperature on the haemolytic activity of *Alcaligenes faecalis*

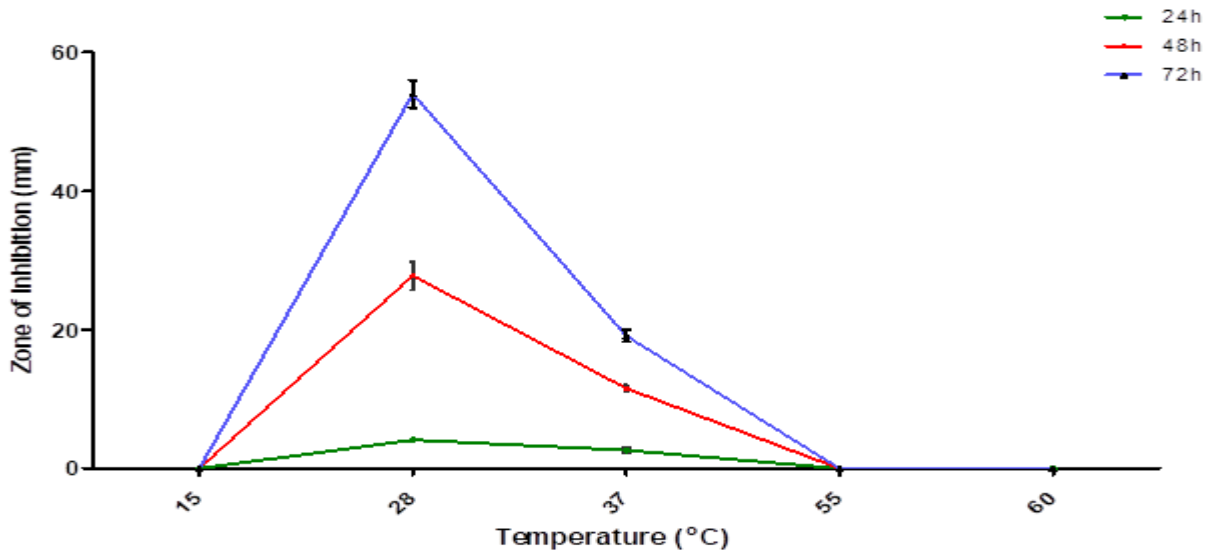


Figure 3. Effect of temperature on haemolytic activity of *Bacillus cereus*

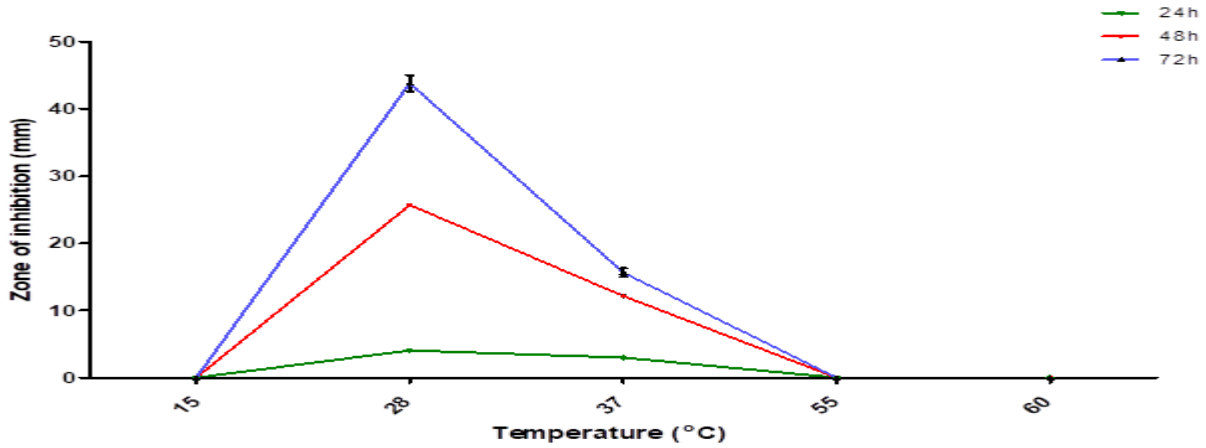


Figure 4. Effect of temperature on the haemolytic activity of *Proteus penneri*

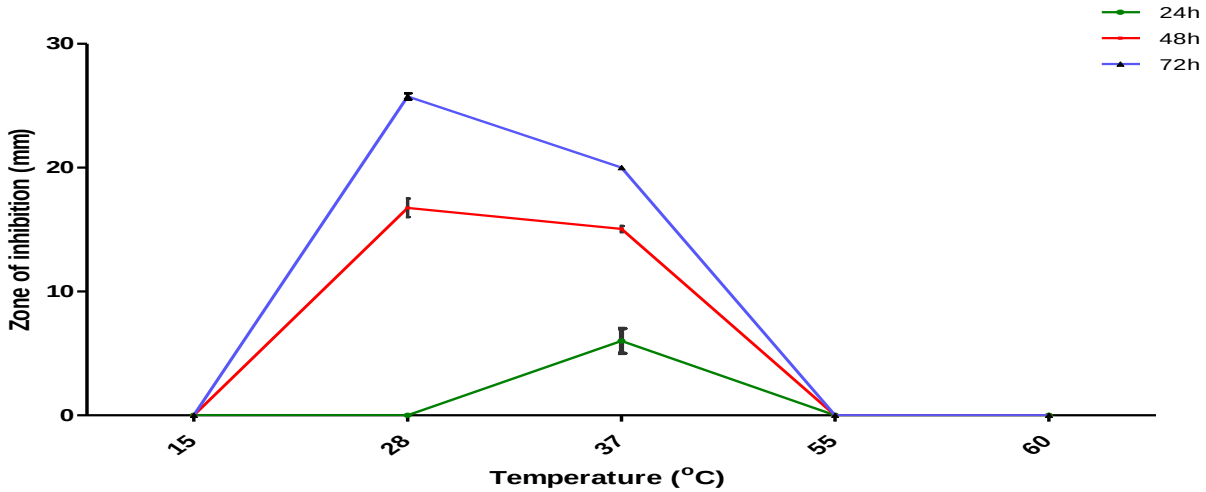


Figure 5. Effect of pH on the haemolytic activity of *Stenotrophomonas* ***acidaminiphilis***

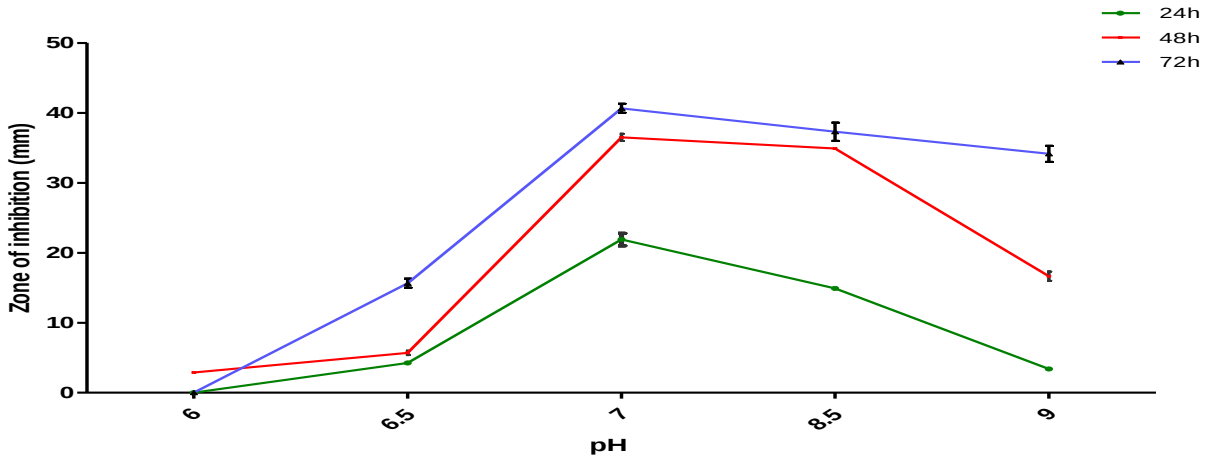


Figure 6. Effect of pH on the haemolytic activity of *Proteus penneri*

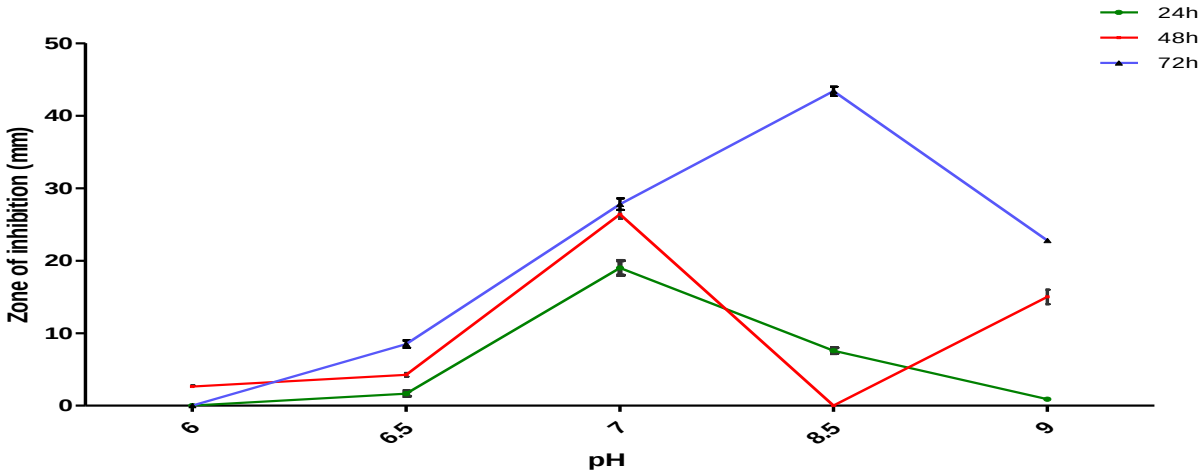


Figure 7. Effect of pH on the haemolytic activity of *Alcaligenes faecalis*

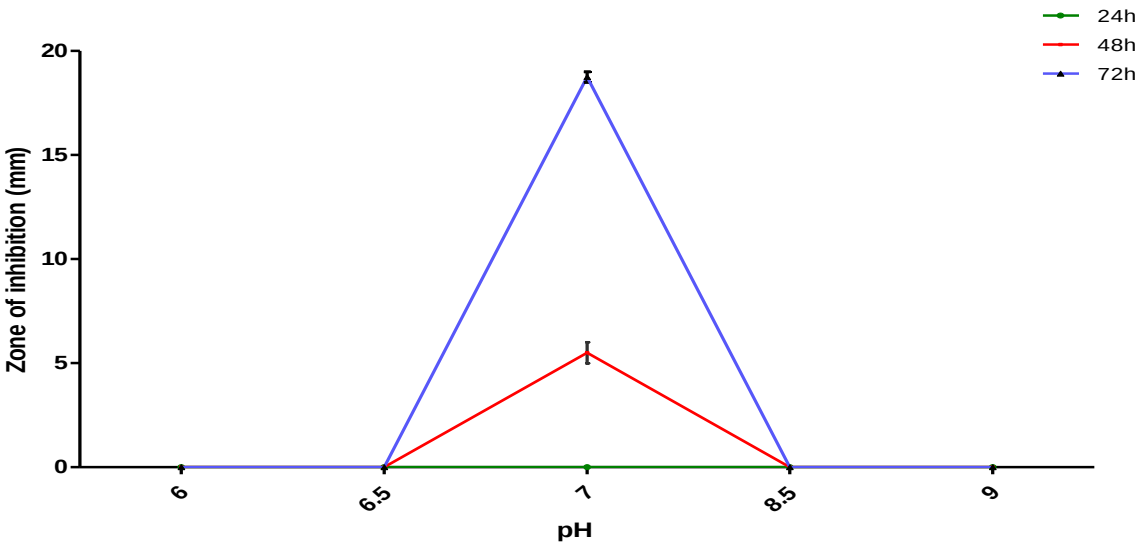


Figure 8. Effect of pH on the haemolytic activity of *Bacillus cereus*

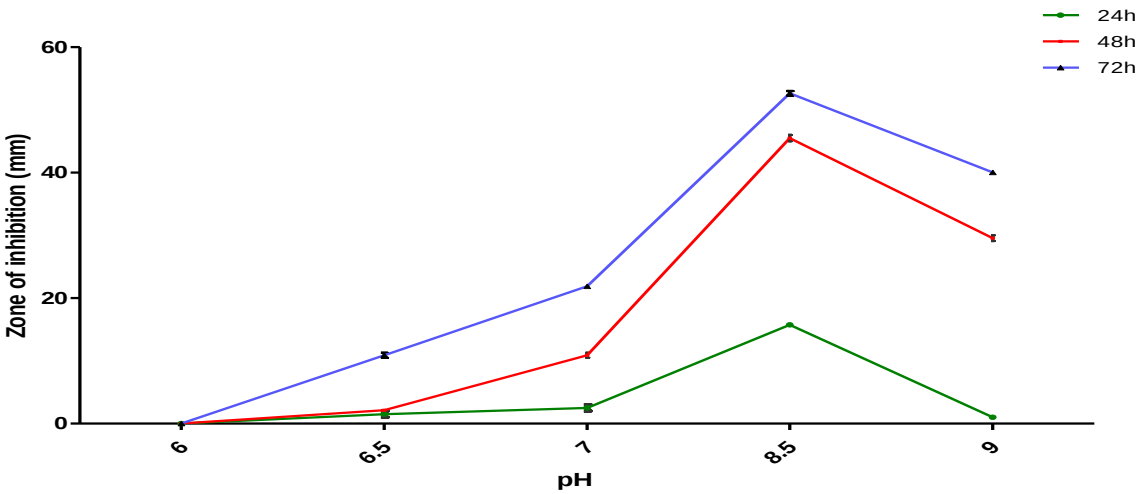


Plate 1. Growth of *Stenotrophomonas acidiminiphilis* on blood agar and occurrence beta-haemolysis at a temperature of 37 ºC for duration of 24 h.

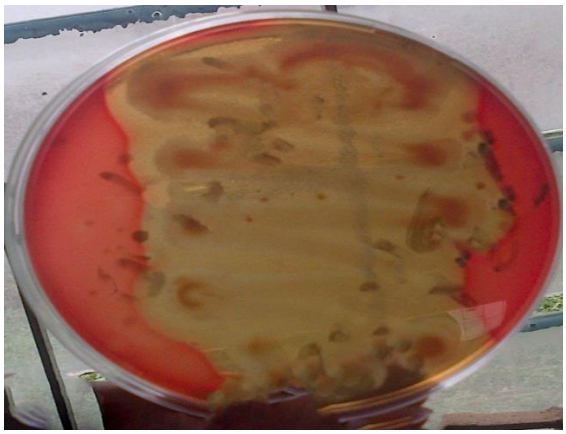
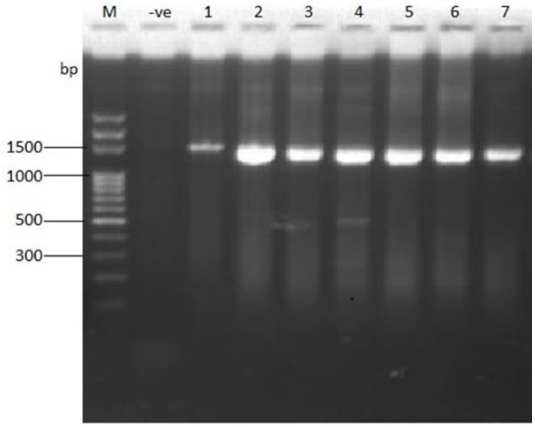


Plate 1. Polymerase chain reaction (PCR) product of electrophoresis gel image showing the DNA fragments of bacterial isolates subjected to 16 Svedberg RNA detection.



Conclusion

The finding affirmed the occurrence of microorganisms especially haemolytic organisms in the stream water samples analysed in this study alluding to the fact that it poses serious threat not only to human health but also to animals and since these organisms can break down red blood cells, they are considered pathogenic. The public should however be informed of the dangers associated with surface waters. Members of the public should be enlightened on the imminent dangers and threat that stream water sources pose especially on the dangers of haemolysis occurring in the human body system for persons who utilized the water for domestic activities. Stream water sources should be treated from time to time and the maintenance of these water bodies should be monitored by regulating bodies and it is highly recommended that human activities such as farming, swimming, washing and bathing in and around the river bank should be reduced or totally discouraged so that the quality of water would be improved.

Acknowledgement

All authors appreciate the output of laboratory technicians at the Department of Microbiology, Federal University of Technology Akure (FUTA) for the success of the study.

Authors' contributions

Author Olusola-Makinde, O. O designed the study. Author Somefun, A. C developed the methodology and acquire the data, analyse the data and interpreted the data. Author Bayode, M. T wrote the manuscript. Olusola-Makinde, O. O corrected and Bayode, M. T fine-tuned the manuscript. All authors read and approved the final manuscript.

Conflict of interest: None.

Funding: No funding received.

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