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Laboratory diagnosis and treatment of pathogenic bacteria isolated from children with cervical lymphadenopathy at Tanta University Hospital

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ABSTRACT

Immune illness, cancer, and microbial infections can cause lymphadenopathy, which enlarges lymph nodes. Current research focuses on laboratory diagnosis of microbial infections that cause lymphadenopathy in children by isolating and identifying microorganisms from throat and lymph node aspiration, performing throat and lymph node aspiration cultures, and treating the most common isolated bacterial infections with bioproducts. Children shown lymphadenopathy were microbiologically examined. Bacterial taxa recovered from cervical lymphadenopathy patients were *Staphylococcus aureus* (MRSA) and *Streptococcus pyogenes*. The two isolates were identified molecularly, and their sequences had been deposited in GenBank under the accession numbers PP527743 and PP527744 respectively. The agar well diffusion test assessed the antimicrobial properties of the clove, garlic, thyme oils and *Aloe vera* gel. Comparatively, garlic oil was the best antibacterial bioproduct against isolated bacteria, with inhibition zones of 20- and 21-mm. respectively. The minimum garlic inhibitory concentrations against MRSA and *S. pyogenes* were 6.25% and 0.19%. Cefotaxime antibiotic came first in comparison with other used antibiotics (Vancomycin, Ampicillin/sulbactam, Penicillin G) against common isolated bacteria and the mean diameter of inhibition zones were 31 mm and 35 mm, respectively. This study concluded that, the most common isolated bacterial infections from children with cervical lymphadenopathy were methicillin-resistant *S. aureus* and *S. pyogenes*. Among the several bioproducts tested against bacteria recovered from children with cervical lymphadenopathy, garlic oil demonstrated the highest antibacterial efficacy.

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Introduction

The lymphatic system is made up of lymph vessels and lymphatic organs such as the thymus, tonsils, lymph nodes, and spleen. These substances contribute to acquired and innate immunity, filter and drain interstitial fluid, and recycle cells at the end of their life cycle (Bujoreanu & Gupta 2024). Lymphadenopathy is enlargement of lymph nodes that can be caused by bacterial, viral, or fungal infections, autoimmune diseases, and malignancies (Maini & Nagalli 2023).

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Cervical lymphadenopathy associated with upper respiratory tract infections can be caused by viruses like rhinovirus, parainfluenza virus, influenza virus, respiratory syncytial virus, coronavirus, adenovirus or reovirus. Other viruses associated with cervical lymphadenopathy involve Epstein-Barr virus (EBV), cytomegalovirus, rubella, rubella, varicella-zoster virus, herpes simplex virus (HSV), coxsackie virus, and human immunodeficiency virus (HIV). Bacterial cervical lymphadenitis is usually caused by group A hemolytic *Streptococcus* or *Staphylococcus aureus*. Anaerobic bacteria can lead to cervical lymphadenitis, which is often associated with tooth decay and periodontal disease. Group B streptococci and *Haemophilus influenzae* type B are less common causative organisms. Diphtheria is a rare cause. *Bartonella henselae*, atypical mycobacteria and mycobacteria are significant contributors to subacute or chronic cervical lymphadenopathy (Spyridis et al. 2001).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most effective modern pathogens. The same commensal organisms transmitted both in health care facilities and in the community are the main reason of bacteremia, endocarditis, skin and tissue infections, soft tissue, and bone and joint infections. and hospital-acquired infections. Genetically diverse, the epidemiology of MRSA is mostly distinguished by the mass emergence of epidemic strains. Although incidence has recently decreased in some areas, MRSA continues to be a serious clinical hazard with morbidity and mortality rates remaining high (Turner et al. 2019). A previously healthy 15-year-old girl was referred to the hospital because of fever and lymphadenopathy in the right axillary, ulnar, and inguinal regions. Methicillin-resistant *Staphylococcus aureus* was detected in all swollen lymph node samples (Eda et al. 2023).

Streptococcus pyogenes is a primarily pathogenic bacterium in humans, causing a variety of manifestations, ranging from mild localized infections to life-threatening invasive infections. Ineffective treatment of *S. pyogenes* infection can lead to post-infectious sequelae of rheumatic fever and post-streptococcal glomerulonephritis. Additionally, it causes invasive infections such as necrotizing fasciitis and toxic shock syndrome that are associated with high morbidity and mortality. *S. pyogenes* is a gram-positive, catalase-negative, oxidase-negative β-hemolytic *Streptococcus*. It is a facultative anaerobe, grows best in 5-10% carbon dioxide and forms spot colonies on blood agar plates. The Lancefield serological subtyping system is employed to distinguish group A streptococci (GAS) from other *Streptococci* (Ibrahim et al. 2016). Sore throat is often the main symptom of strep throat. The most common clinical signs of streptococcal pharyngitis include sudden onset of fever, malaise, pharyngeal discharge, cervical lymphadenopathy, and tonsillitis (Ebell et al. 2000). The increasing number of multidrug-resistant and antibiotic-resistant bacteria leads to a continued need to find alternative treatments for infections. Thyme, clove, and cinnamon bark essential oils may have promising antibacterial activity against *S. pneumoniae* and *S. pyogenes* (Ács et al. 2018).

It is well known that essential oils (EOs) are known for their biological activities, especially their antibacterial properties. In a previous study, EOs from seven aromatic plants from the Asir area of southwestern Saudi Arabia were tested for antibacterial efficacy against four drug-resistant strains of pathogenic bacteria (*Staphylococcus aureus* & *Streptococcus pyogenes*) (Helal et al. 2019). The antibacterial effect of garlic has been studied against 133 strains of multidrug-resistant Gram-positive and negative bacteria (Iwalokun et al. 2004).

This study was designed for laboratory diagnosis of microbial infections that can cause lymphadenopathy in children and treatment the most common isolated microbial infections by using some bio products (Clove oil & Garlic oil & Thyme oil & Aloe Vera gel).

Materials and Methods

Sampling

Throat swab and lymph node aspiration samples were taken from children with lymphadenopathy (35 children; 22 males and 13 females) (their age ranged from 2 to 13 years old) at the Hematology unit, Tanta University Hospital. Throat swabs were taken from healthy control children (20 children; 11 males and 9 females) (their age ranged from 2 to 13 years old). The throat swab and lymph node aspiration samples subjected to processing for isolation and identification of microorganisms. This study was done according to guidelines of Ethics Committee of Scientific Research for medical research at Tanta university with code # 32017/02/18.

Identification of Methicillin Resistant Staphylococcus aureus (MRSA)

The samples were inoculated onto nutrient agar, blood agar, mannitol salt agar, oxacillin resistance screening agar base (ORSAB) and then incubated at 37 °C for 24 hours. The isolated bacteria were investigated by microscope after Gram stain. The Gram positive staphylococci tested to catalase and coagulase test and other biochemical tests (Berke & Tilton, 1986; Becker et al. 2002).

Identification of Streptococcus pyogenes

The samples were inoculated onto nutrient agar, blood agar, and incubated at 37 °C for 24 hours. Microscopically, isolated bacteria were investigated after Gram stain. The Gram positive beta hemolytic streptococci tested to catalase and bacitracin antibiotic sensitivity and other biochemical tests (Levinson & Frank 1955).

Identification of isolated bacteria by VITEK 2 Compact system

The isolated bacteria were directly identified by the VITEK 2 compact (bioMerieux Inc. USA) system ´using GP ID REF21342 (Identification-Gram-positive bacteria) cards according to Aubertine et al. (2006).

Molecular Identification of isolated bacteria by 16S ribosomal RNA gene segments analysis

DNA was extracted from isolated bacteria by using Thermo Scientific GeneJET Genomic DNA Purification kit according to Sambrook & Russell (2001). DNA products were subjected to Polymerase chain reaction (PCR) and agarose gel electrophoresis Followed by Purification of DNA fragments from the gel. DNA fragments were then subjected for DNA sequencing according to Sanger et al. (1977) and Tabor & Richardson (1995). Sample analyses were made by OpenGene software Version 3.1 from Visible Genetics, Canada at The Regional Center for Mycology and Biotechnology. Primer sequences used for the identification of 16s in the current study are:

fD15 (5'- AGA GTT TGA TCC TGG CTC AG-'3)

rP25 (5'- ACG GCT ACC TTG TTA CGA CTT-'3)

Antimicrobial activity of some bioproducts against the isolated bacteria by agar well diffusion method

In the present study four bioproducts (*Aloe vera* gel, *Allium sativum* oil, *Eugenia caryophyllata* oil and *Thymus vulgaris* oil) were purchased from El-Hawag company, Badr city, Egypt and tested for their antimicrobial activities at Mueller Hinton agar media against two common isolated bacteria Methicillin resistant *Staphylococcus aureus* and *S. pyogenes* at concentration of 1 $x10^6$ cfu/ml by the agar diffusion technique according to Perez et al. (1990).

The tested bioproducts were dissolved in dimethyl sulfoxide (DMSO), and 100 µl of each bioproduct were used and 100 µl DMSO was used as control. Three replicates were made for each test. All plates were incubated at 37 ºC for 48h. Then the average diameters of the inhibition zones (mm) were measured.

Determination of minimum inhibitory concentration (MIC) of some essential oils against the isolated bacteria

Each bioproduct was serially diluted by DMSO to give concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56 %, 0.78 %, 0.39 %, 0.19 % etc and examined to obtain minimum inhibitory concentrations (MICs) according to Jennifer (2001).

Antibiotic sensitivity test against the common isolated pathogenic bacteria by agar disc diffusion method

For the Kirby-Bauer disc diffusion test (Bauer et al. 1966), four antibiotics, namely Vancomycin, Cefotaxime, Ampicillin/sulbactam, and Penicillin G, were employed as positive controls to evaluate their effectiveness against the isolated bacteria.

Results

Methicillin resistant *Staphylococcus* and *Streptococcus pyogenes* were isolated from children with cervical lymphadenopathy. MRSA was detected in 22% of children with lymphadenopathy. *S pyogenes* was detected in 9.1% of children with lymphadenopathy. Healthy control children were negative to any pathogenic bacteria. As shown in table (1), the percentage of male in children with lymphadenopathy were 62.9 % and females were 37.1%%. The percentage of children with cervical lymphadenopathy were 88.6 %, the percentage of children with abdominal lymphadenopathy were 8.6 % and finally the percentage of children with Axillary lymphadenopathy were 2.8%.

The results revealed the presence of Methicillin-resistant *Staphylococcus aureus* (MRSA) as expressed in Figs (1 & 2). The identification of Methicillin-resistant *Staphylococcus aureus* confirmed by biochemical tests and 16 s ribosomal RNA sequencing.

As shown in figure (2) and table (2), MRSA gave positive results in catalase test and coagulase test but negative results in oxidase test. The identification of methicillin resistant Staphylococcus aureus is confirmed by vitek2 compact system.

The present experiment aimed to investigate the presence of microbial infections in children with cervical lymphadenopathy using throat culture. The results represented in Fig. 3 and Table 2 revealed the presence of S. pyogenes as gramme-positive cocci with white co colony on nutrient agar with beta hemolysis in blood agar, and there was no effervescence of bubbles during exposure to H2O2 in the catalase test. The identification of S. pyogenes was confirmed by biochemical tests and 16S ribosomal RNA sequencing.

As shown in table (2), *S. pyogenes* gave sensitive to bacitracin antibiotic but negative results in catalase test. The identification of *S. pyogenes* is confirmed by vitek2 compact system.

DNA was successfully extracted from two isolates as indicated by the Agarose gel electrophoresis, which showing the PCR amplified DNA product as distinct bands for each isolate (*S. aureus & S. pyogenes*). From the PCR products, the approximated molecular sizes of the amplicons were about 1500 bp (Fig. 4). The two isolates sequences had been deposited in GenBank under the accession numbers (Table 3)**.** Phylogenetic tree based on 16S rRNA sequences for the two isolates represented in figures 5 & 6.

Table 1 Percentage of males and females among children with lymphadenopathy and types of lymphadenopathies

Sex	N	%	Types of lymphadenopathy	N	%
Male	22	62.9	Cervical Lymphadenopathy	31	88.6
Female	13	37.1	Abdominal lymphadenopathy	3	8.6
Total	35	100	Axillary lymphadenopathy	1	2.8

N: Number; %: percent

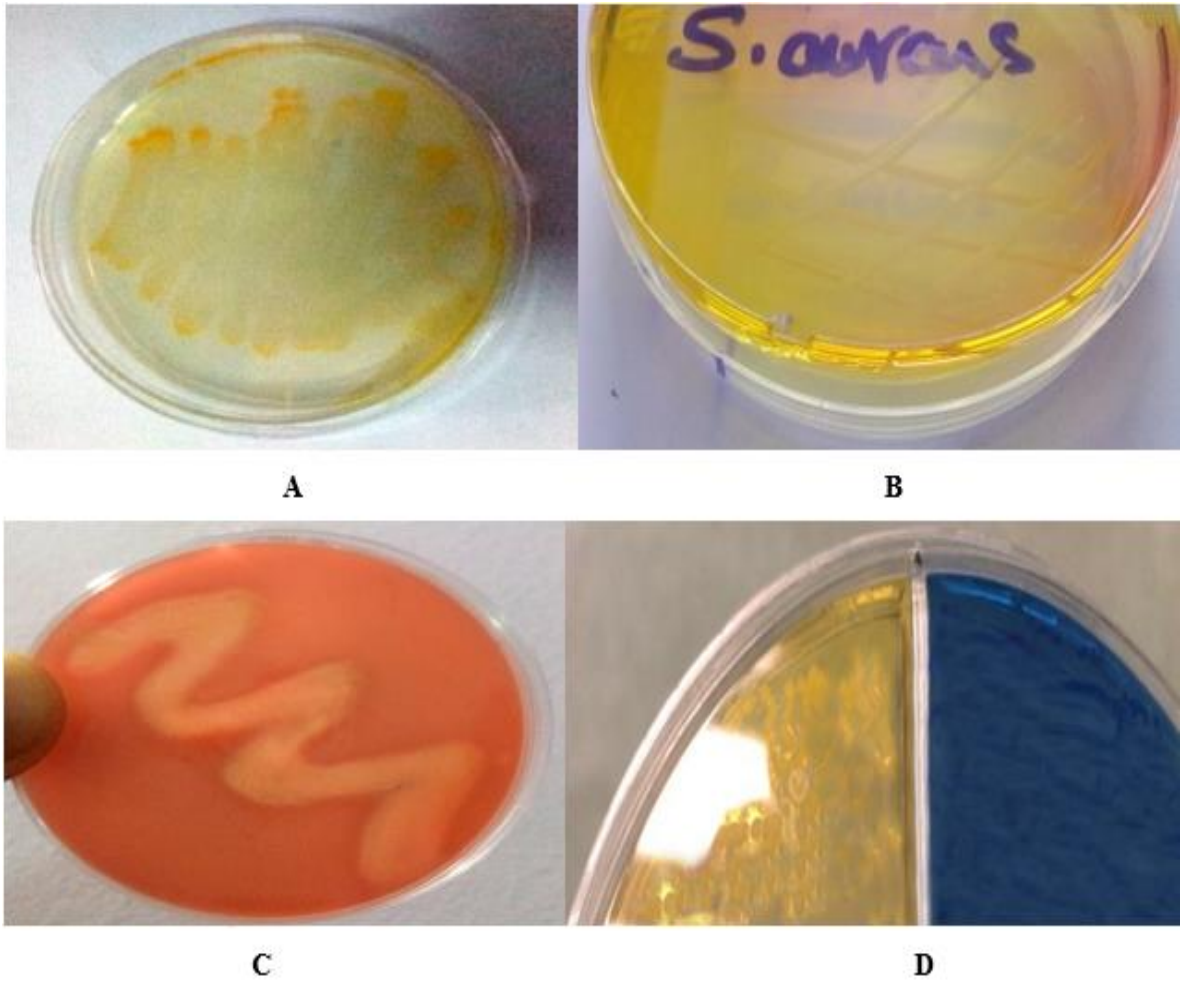

Fig. 1 Methicillin - resistant *Staphylococcus aureus* isolated from children with cervical lymphadenopathy. A: on nutrient agar (N agar), B: on mannitol salt agar (MSA), C: on Blood agar, D: on ORSAB

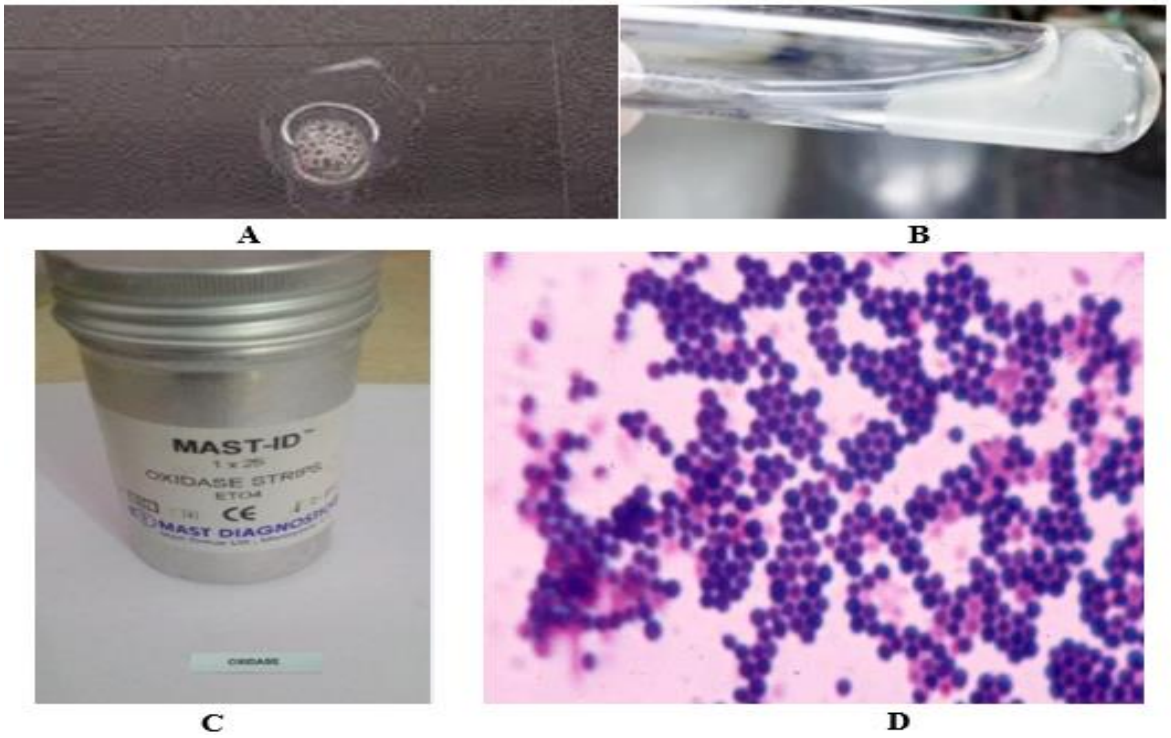

Fig. 2 Identification on Methicillin-resistant *Staphylococcus aureus* by Biochemical tests. A: Positive catalase test, B: Positive coagulase test, C: Negative Oxidase test, D: MRSA stained by Gram stain

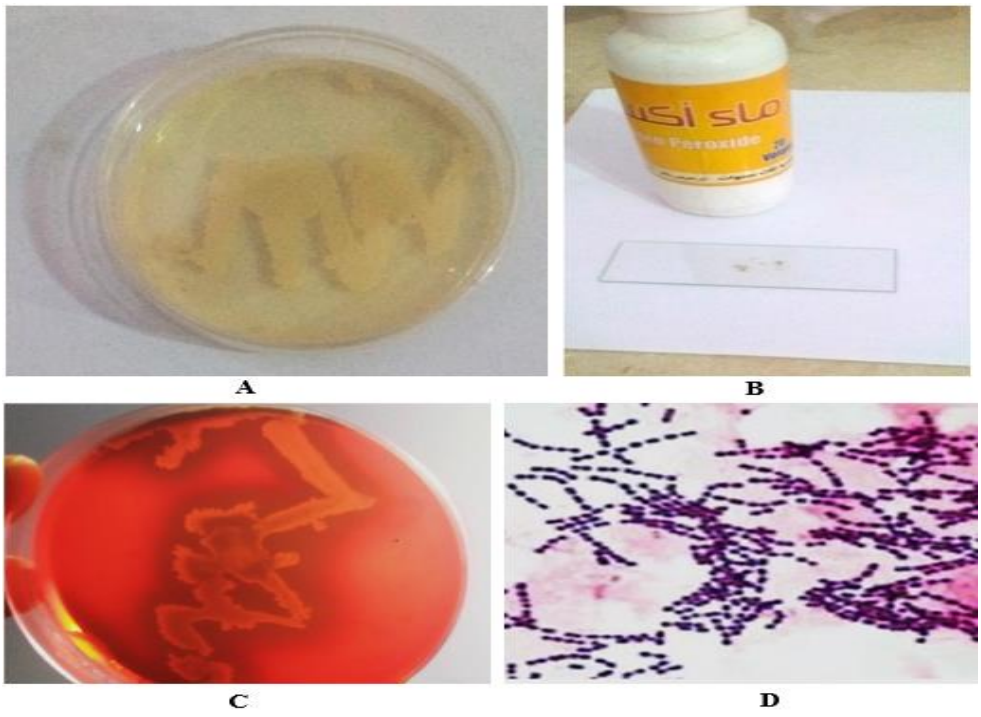

Fig. 3 (A) S. *pyogenes* on nutrient agar & (B) Negative catalase test & (C) *pyogenes* on Blood agar & (D) *S pyogenes* stained by gram stain

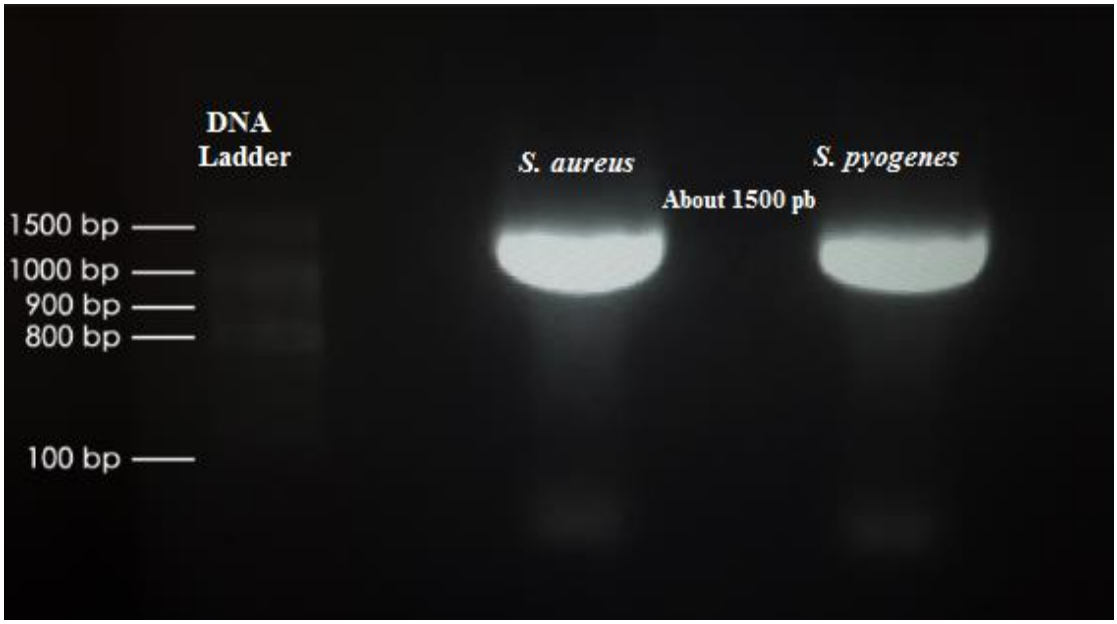

Fig. 4 Agarose gel electrophoresis showing the PCR amplified DNA product obtained from the bacterial isolates

Table 2 Identification of isolated bacteria by biochemical tests

S. aureus		*S. pyogenes*	
Tests	**Result**	**Test**	**Result**
Gram stain	Positive (+Ve)	Gram stain	Positive
Shape under microscope	grape-like clusters	Shape under microscope	In chains
Oxidase test	Negative (-Ve)	Catalase	Negative
Catalase test	Positive (+Ve)	Blood agar	Beta hemolytic
Coagulase	Positive (+Ve)	Sensitivity to Bacitracin	Sensitive
Citrate	Positive (+Ve)	Motility	Negative (-Ve)
Gelatin Hydrolysis	Positive (+Ve)	Pyrrolidonyl-β-naphthylamide (PYR)	Positive (+Ve)
H2S	Negative (-Ve)	Urease	Negative (-Ve)
MR (Methyl Red)	Positive (+Ve)	Voges Proskauer (VP)	Negative (-Ve)
Nitrate Reduction	Positive (+Ve)	Hyalurodinase	Positive (+Ve)
Urease	Positive (+Ve)	Arginine Dehydrolase	Positive (+Ve)
Voges Proskauer (VP)	Positive (+Ve)	Fermentation of Adonitol	Negative (-Ve)
Pyrrolidonyl-β-naphthylamide (PYR)	Negative (-Ve)	Fermentation of Arabinose	Negative (-Ve)
Motility	Negative (-Ve)	Fermentation of Arabitol	Negative (-Ve)
Lipase	Positive (+Ve)	Fermentation of Fructose	Positive (+Ve)
Hyalurodinase	Positive (+Ve)	Fermentation of Galactose	Positive (+Ve)
Arginine Dehydrolase	Positive (+Ve)	Fermentation of Glucose	Positive (+Ve)
Fermentation of Arabinose	Negative (-Ve)	Fermentation of Lactose	Positive (+Ve)
Fermentation of Cellobiose	Negative (-Ve)	Fermentation of Maltose	Positive (+Ve)
Fermentation of Fructose	Positive (+Ve)	Fermentation of Mannitol	Negative (-Ve)
Fermentation of Galactose	Positive (+Ve)	Fermentation of Melibiose	Negative (-Ve)
Fermentation of Glucose	Positive (+Ve)	Fermentation of Raffinose	Negative (-Ve)
Fermentation of Lactose	Positive (+Ve)	Fermentation of Ribose	Negative (-Ve)
Fermentation of Maltose	Positive (+Ve)	Fermentation of Sorbitol	Negative (-Ve)
Fermentation of Mannitol	Positive (+Ve)	Fermentation of Sucrose	Positive (+Ve)
Fermentation of Mannose	Positive (+Ve)	Fermentation of Trehalose	Positive (+Ve)
Fermentation of Raffinose	Negative (-Ve)	Fermentation of Xylose	Negative (-Ve)
Fermentation of Ribose	Positive (+Ve)		
Fermentation of Sucrose	Positive (+Ve)		
Fermentation of Trehalose	Positive (+Ve)		
Fermentation of Xylose	Negative (-Ve)		

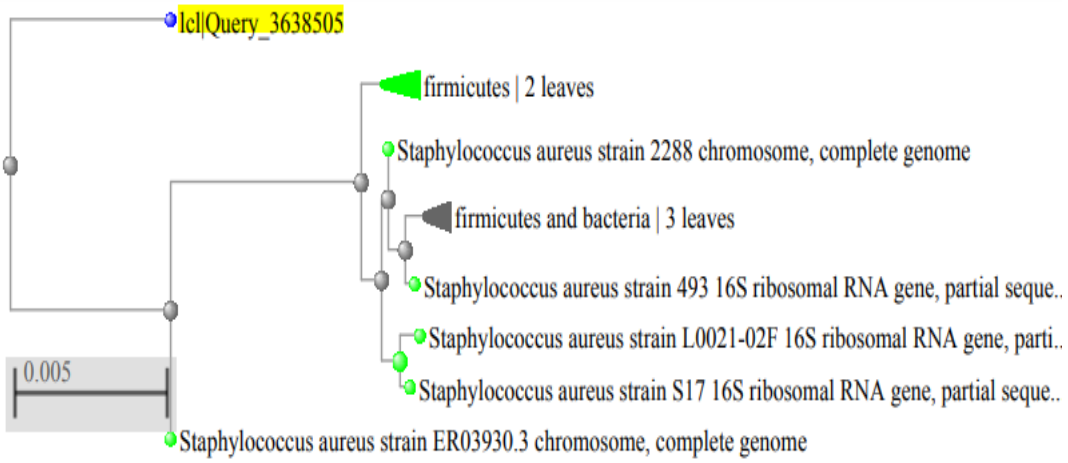

Fig 5. Phylogenetic tree based on 16S rRNA sequences for MRSA.

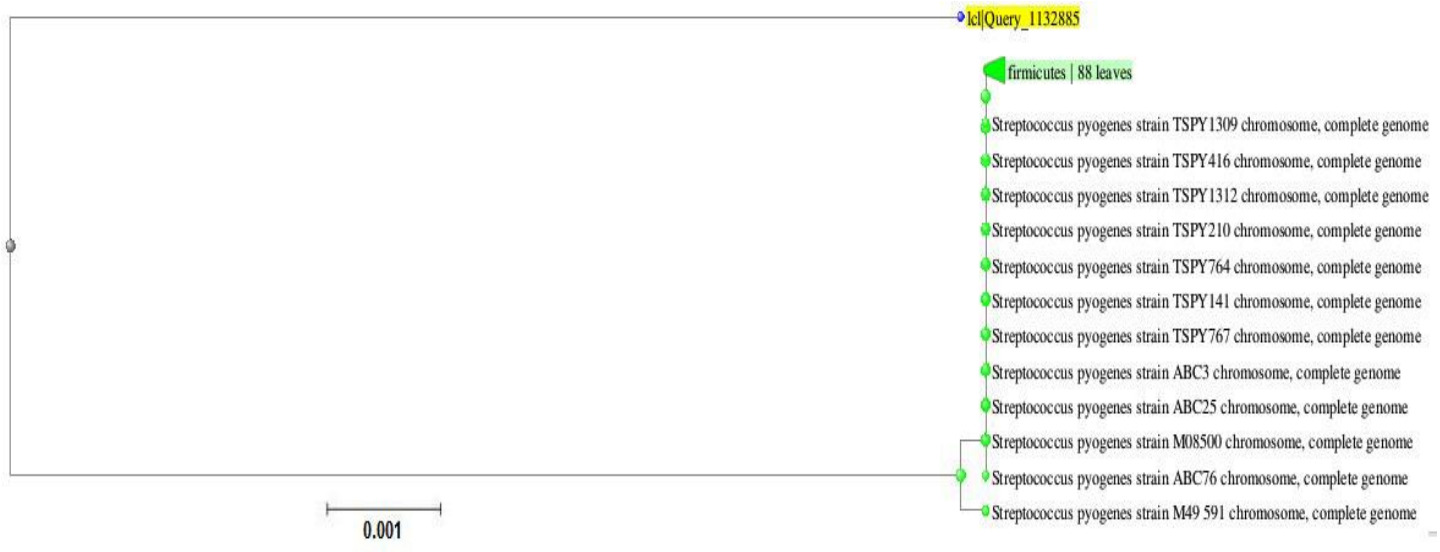

Fig 6. Phylogenetic tree based on 16S rRNA sequences for *Streptococcus pyogenes.*

Table 3 GenBank Accession Numbers of DNA Sequences of isolated bacteria included in this study

GenBank Accession Numbers	Identified strains
PP527743	*Staphylococcus aureus* (Ahmedsamy1)
PP527744	*Streptococcus Pyogenes* (Ahmedsamy2)

Tests were done on some bioproducts to see how well they killed common pathogenic bacteria like Methicillin-resistant S. aureus and S. pyogenes. As shown in table (4) and figures 7 and 8, garlic oil was the most effective.

The lowest concentrations of *Aloe vera* gel, clove oil, garlic oil, and thyme oil that were effective in killing *S. pyogenes* were 6.25%, 0.19%, 0.19%, and 0.19%, respectively (Table 5). The lowest concentrations of the bioproducts that were effective in killing MRSA were 50%, 1.56%, 6.25%, and 0.78%, respectively.

Vancomycin, Cefotaxime, Ampicillin/sulbactam, and Penicillin G were tested for their antimicrobial activity against common isolated pathogenic bacteria (Methicillin-resistant *S. aureus* and *S. pyogenes*). Cefotaxime exhibited the most potent antimicrobial activity (Table 6, Figs 9 & 10).

Table 4 Inhibition zones (mm) of different bioproducts against common isolated bacteria

Isolated bacteria	*Aloe vera* gel	Clove oil	Garlic oil	Thyme oil
S. pyogenes	16	18	21	14
MRSA	14	17	20	19

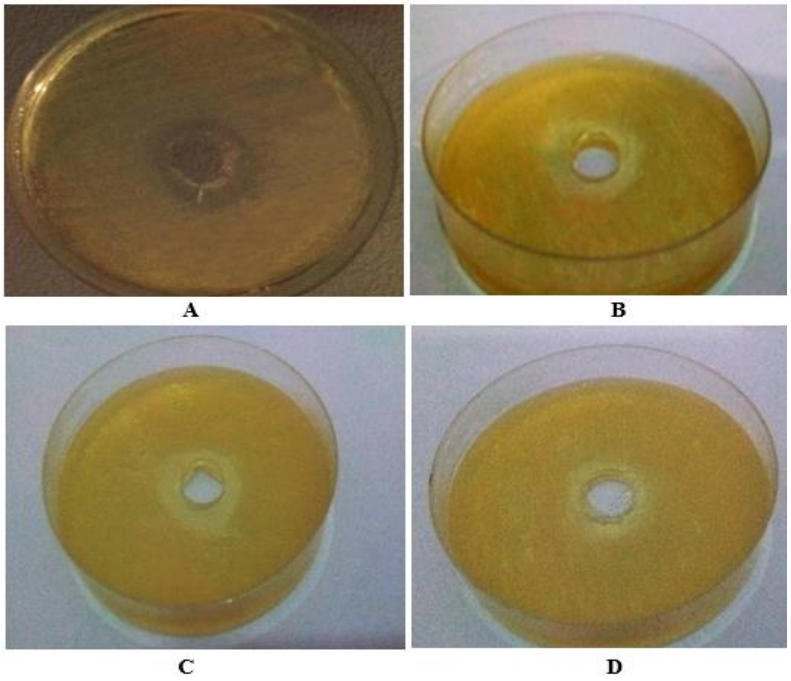

Fig. 7 Inhibition zones of different bioproducts on *Streptococcus pyogenes.* A. *Aloe vera* gel; B. Clove oil; C. Garlic oil; D. Thyme oil.

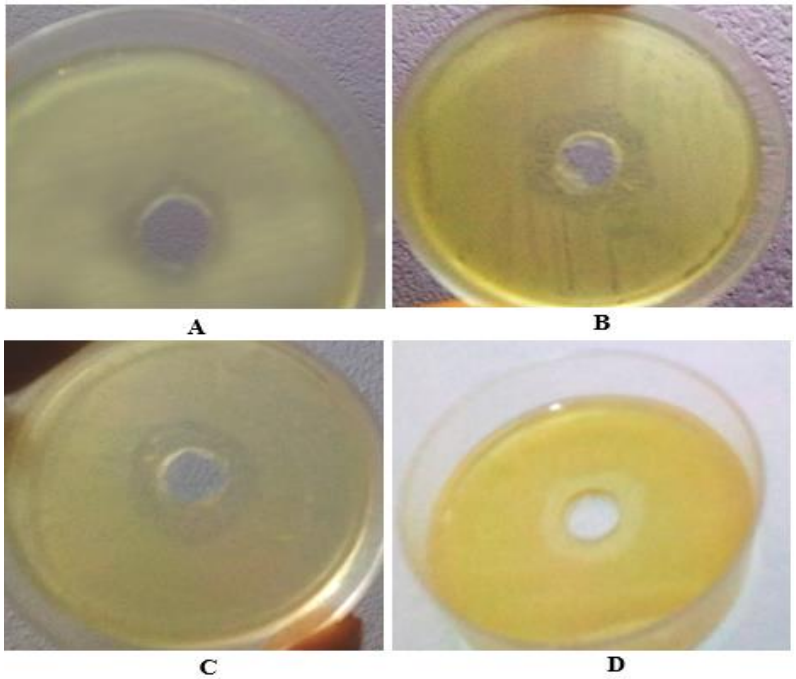

Fig. 8 Inhibition zones of different bioproducts on methicillin Resistant *S. aureus.* A. *Aloe vera* gel; B. Clove oil; C. Garlic oil; D. Thyme oil.

Table 5 Minimum inhibitory concentrations of different bio products against common isolated bacteria

Parameters	Aloe Vera gel	Clove oil	Garlic oil	Thyme oil
S. Pyogenes	6.25 %	0.19%	0.19%	0.19%
MRSA	50%	1.56%	6.25%	0.78%

Table 6 Inhibition zones (mm) of some antibiotics against common isolated bacteria

Bacteria	Vancomycin (VA) (30 µg/disk)	Cefotaxime (CTX) (30 µg/disk)	Ampicillin/sulbactam (AX) (10/10 µg/disk)	Penicillin G (P) (10 /UI)
S. pyogenes	22	35	24	28
MRSA	21	31	27	Resistant

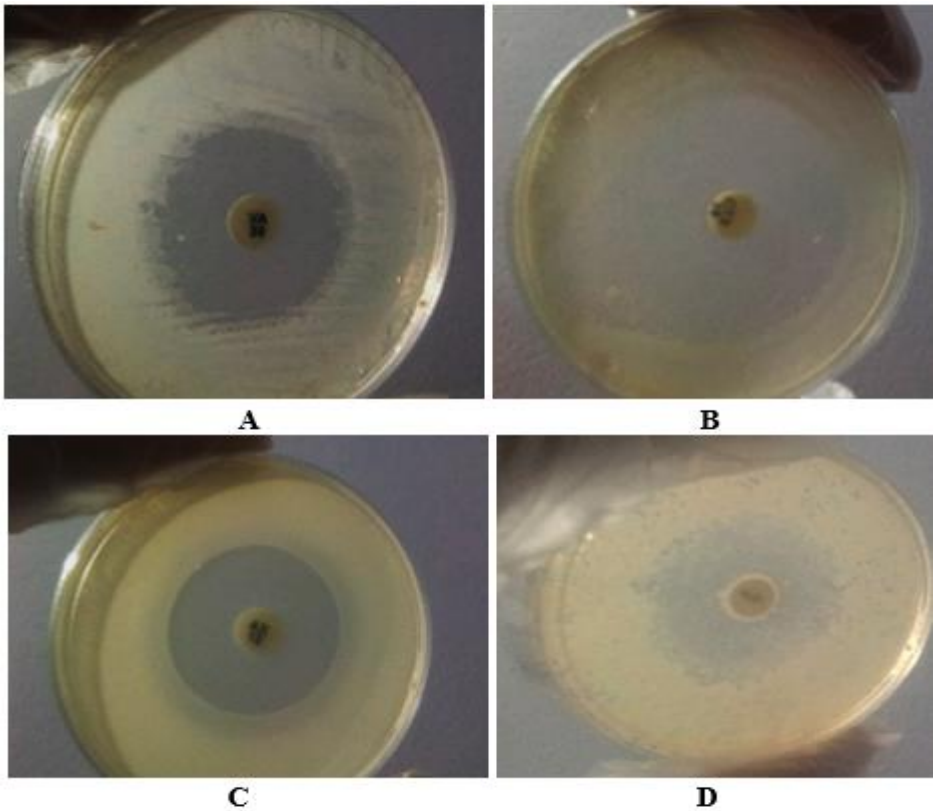

Fig. 9 Inhibition zones of some antibiotics against *Streptococcus pyogenes*. A. Vancomycin (30 µg/disk); B. Cefotaxime (30 µg/disk); C. Ampicillin/sulbactam (10/10 µg/disk); D. Penicillin G (10 /UI).

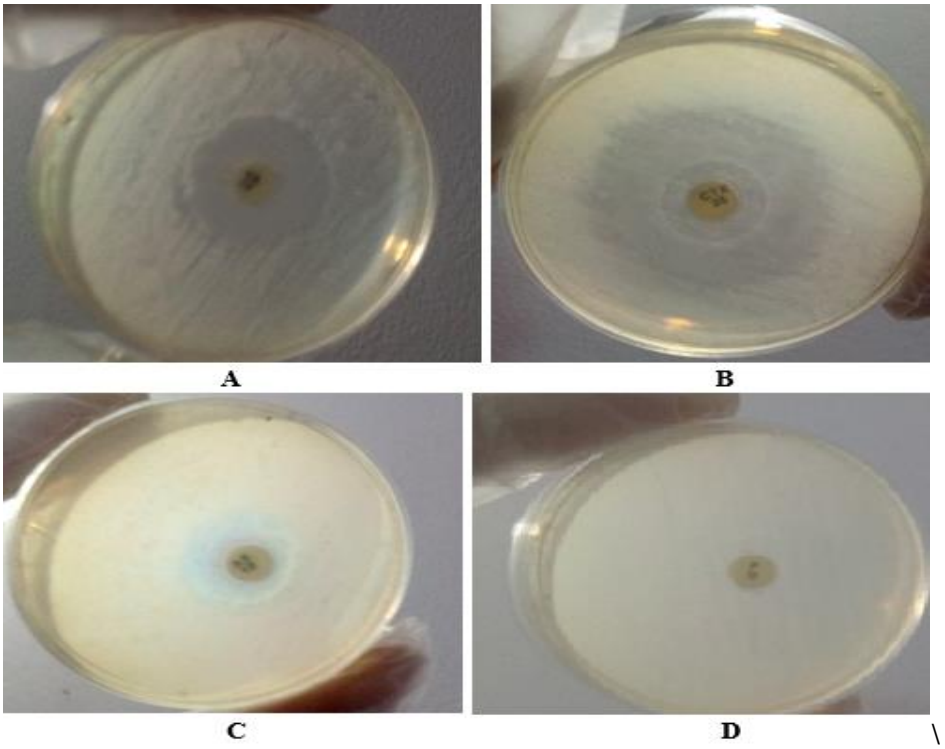

Fig. 10. Inhibition zones of some antibiotics against MRSA. A- Vancomycin (30 µg/disk); B- Cefotaxime (30 µg/disk); C- Ampicillin/sulbactam (10/10 µg/disk); D- Penicillin G (10 /UI).

Discussion

Lymphadenopathy is an irregularity in the size and texture of the lymph nodes, which is quite common in childhood. When the enlargement of lymph nodes is caused by inflammatory and infectious processes, it is called lymphadenitis (Pecora et al. 2021).

In the present study, the obtained results indicated isolation of methicillin-resistant *Staphylococcus aureus* and *Streptococcus pyogenes* from children with cervical lymphadenopathy by throat culture and lymph node aspiration culture.

In the same context, Ahonkhai et al. (1984) reported that; Sixty-one cases of acute cervical lymphadenitis in Kings County Hospital Center were reviewed. *Staphylococcus aureus* accounted for 50 percent of the cases and Group A β-hemolytic *streptococcus* accounted for 15 percent; 35 percent had no growth on culture. The obtained results are with the same line with those obtained from Reddy et al. (2002) who studied cervical lymphadenitis. They reported that acute bilateral cervical lymphadenitis is usually caused by a viral upper respiratory tract infection or streptococcal pharyngitis. Acute unilateral cervical lymphadenitis is caused by streptococcal or staphylococcal infection in 40% to 80% of cases. Common causes of subacute or chronic lymphadenitis include cat-scratch disease and mycobacterial infection. Generalized lymphadenopathy is often caused by a viral infection, and less frequently by malignancies, collagen vascular diseases, and medications. In contrast to results obtained from Rombaux et al. (2000) who reported that, *Bartonella henselae* was linked to children and young adults with lymphadenopathy. Surgical excision of the lymphadenopathy is useful to establish the diagnosis when serology is not available and/or when the adenopathy become fluctuating.

In the present work, the obtained results indicated that garlic oil was the best antimicrobial bio products from the other used bio products (thyme oil, clove oil and Aloe vera gel) against common isolated bacteria (Methicillin-resistant *S. aureus & Streptococcus pyogenes*). MICs of garlic oil against *methicillin-resistant S. aureus* and *S. pyogenes* were 6.25 % , 0.19% respectively and diameter of inhibition zones were 20 mm and 21 mm respectively.

These results were consistent with Tsao and Yin (2001) who reported that, there was antimicrobial activity of garlic oil, Chinese leek oil against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), three Candida spp. and three Aspergillus sp. In similar study Savitri, et al. (2019) reported that Garlic (*Allium sativum*) has an effect of bactericidal activity, it can perform as an antibacterial for *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. Bhatwalkar et al. (2021)

reported that garlic (*Allium sativum*) has been used traditionally to treat various ailments especially bacterial infections for centuries in various cultures around the world. The principal phytochemicals that exhibit antibacterial activity are oil-soluble organosulfur compounds that include allicin, ajoenes, and allyl sulfides. The organosulfur compounds of garlic exhibit a range of antibacterial properties such as bactericidal, antibiofilm, antitoxin, and anti-quorum sensing activity against a wide range of bacteria including multi-drug resistant (MDR) strains.

In similar study, Abidullah et al. (2021) revealed that garlic makes large clear zones in comparison to the currently available antibiotics used in the study. The potentiality of the garlic can be utilized in the field of antibacterial agents. It can be prepared in the form of tablets in the best concentrations and affordable dosages so that it can be used as medicine against these pathogenic organisms.

Similarly, Magryś et al. (2021) reported that *Allium sativum* possessed antibacterial properties against the most prevalent multidrug-resistant bacteria. As a consequence of the findings, garlic has been recognised as the most effective plant species for the treatment of bacterial infections for long. This study is designed to assess the antibacterial activity of *Allium sativum* extracts and their interactions with selected antibiotics against drug-sensitive and multidrug-resistant isolates of emerging bacterial pathogens that are frequently encountered in healthcare settings, bearing in mind the extensive potential of garlic as a source of antimicrobial drugs.

The in vitro data obtained in this study demonstrated that the entire *Allium sativum* extract inhibited the growth of a diverse spectrum of bacteria, including multidrug-resistant strains with bactericidal or bacteriostatic effects. Additionally, these findings were in accordance with Ibrahim et al. (2016), who investigated the antimicrobial properties of certain essential oils, including thyme, clove, and garlic, against *Staphylococcus aureus*. The count of *S. aureus* was significantly reduced by all of the essential oils that were employed. Furthermore, the results suggested that the bacterial counts decreased as the oil concentration increased.

Conclusion

MRSA and *S. pyogenes* are the most common isolated bacterial infections in children with cervical lymphadenopathy. Garlic oil was the best antimicrobial bioproduct out of the other used bioproducts (thyme oil, clove oil, and aloe vera gel) against common isolated bacteria.

Conflict of interest

The authors declare that they have no conflict of interest.

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