A Practical Report

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WIDESPREAD OF DIPHTHERIA IN AFRICA (NIGERIA) Prevalence of Corynebacteriumdiphtheriae, Identification, Pathogenic Impact and Control

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ABSTRACT

Corynebacteriumdiphtheriae is a gram-positive bacterium that causes diphtheria, a severe and potentially life-threatening. With a mortality rate of up to 20%, diphtheria remains a significant public health concern, particularly in areas like Africa with low vaccination coverage[1]. This will provide an overview of *Corynebacteriumdiphtheriae*pathogenesis, epidemiology, and laboratory practical, as well as diagnosis and treatment options. We will also discuss the history of diphtheria vaccination, current vaccination strategies, and the ongoing efforts to combat this preventable disease. This will highlight the need for sustained public health efforts to maintain high vaccination rates and prevent resurgence of this deadly disease.

Keywords: Diphtheria, Nigeria, Corynebacterium diphtheria, Control

INTRODUCTION

Corynebacteriumdiphtheriae is a gram-positive, rod-shaped bacterium that has been a significant public health concern for centuries.[1,3-13] First identified by German bacteriologist Edwin Klebs in 1883, C. diphtheriae is the causative agent of diphtheria.[13]

It possesses a toxigenic strains that are lysogenic for one of the family of corynebacteriophage that carries structural gene for diphtheria toxins and tox[13]. Toxigenic strains secrete a potent exotoxin which may cause diphtheria, It is a highly infectious and potentially life-threatening disease characterized by the production of a potent toxin that can cause severe respiratory and cutaneous manifestations[7]. *Corynebacterium diphtheria* infects the nasopharynx or skin. Diphtheria is most commonly an infection of the upper respiratory tract and causes fever, sore throat, and malaise.

A rich history dating back to ancient civilizations, diphtheria has left an indelible mark on human health, prompting widespread vaccination efforts and ongoing research to combat this preventable disease. Despite significant progress in controlling the disease, *Corynebacterium diphtheriae* remains a formidable pathogen, necessitating continued vigilance and public health efforts to prevent its resurgence [8-9].

According to the Nigerian Centre for Disease Control (NCDC) and WHO, there have been 13 416 suspected cases since May 9, 2022, with 8576 confirmed cases across 116 Local Government Areas (LGAs) in 19 States and the Federal Capital Territory, as of Oct 1, 2023. The hardest-hit States are Kano, Yobe,

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Katsina, Bauchi, Borno, and Kaduna, accounting for 95·8% of cases, with 73·6% affecting children aged 1–14 years. Kano is the epicentre, reporting about 85% of cases. As of Oct 12, 2023, diphtheria has caused over 600 deaths, primarily in children 12.

MATERIAL USED

LOEFFLERS SERUM SLOPES(CULTURING)

Nutrient broth
Normal horse serum
Glucose

GRAM STAINING TECHNIQUE

Solution 1 ammonium oxolate- crystal violet

Crystal violet 20g
95% ethanol 200ml
Filter before use
Ammonium oxolate 1% aqueous solution 800ml

Solution 2 Lugol's iodine solution

Iodine 10g
Potassium iodide 6g
Distilled water 1000ml

Solution 3 Grams iodine

Iodine 10g
Potassium iodide 6g
Ethanol 90ml
Distilled water 10ml

Solution 4 iodine-acetone

Lugol's iodine 35ml
Acetone 965ml
Counter stain : safranin

CYSTEINASE TEST

Tinsdale base 200ml
Difcotinsdale supplement 15ml
-1 vial + 15ml sterile distilled water

BIOCHEMICAL TEST USING ROSCO IDENTIFICATION SYSTEM

Nitrate reduction diagnostic tablet
Urease diagnostic tablet
Glucose tablet
Maltose tablet
Sucrose tablet

METHODS

Sample collection(one specimen) at the Good health lab Bayelsa state, at 12:30pm. The research was from 5th of June 2024 to 1st of November, 2024 when the research was concluded.

The specimens were derived from routine hematological analyses performed on patient blood samples as part of standard clinical care. These samples were subsequently utilized for further investigation.

Loefflers serum slopes(culturing): To 100ml sterile nutrient agar add 2.0g Glucose and shake to dissolve, place in a steamer for 5 minutes. Remove and cool to 56°c, aseptically transfer 300ml sterile horse serum into a sterile broth and then the cooled nutrient broth plus glucose, mix appropriately. Aseptically fill 3.0ml amount into a sterile bijoux bottle, slope in the inspissator and sterilize by heating for 60 minutes at 75-80°c on two consecutive days. And store at 4°c

Gram staining technique: Prepare and fix by heat slide preparation of suspected *Corynebacteriumdiphtheriae*.Cover slide with solution 1 and allow to act for 30 seconds, Pour off and wash freely with iodine solution 2, cover with fresh iodine solution and allow to act for 30 seconds, Pour off iodine solution and wash freely with iodine-acetone, solution 4, cover with fresh iodine-acetone solution and allow to act for 30 seconds, Wash thoroughly with water and Counterstain with safranin, Wash with water, blot and dry.

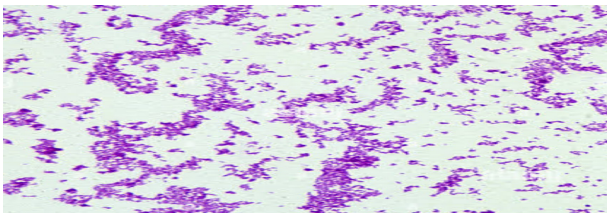

Fig- 1 Corynebacterium diphtheria under counterstaining by safranin * 100

Cysteinase test: Demarcate a small section on a Tinsdale agar plate as the positive control area. Inoculate this area with a known Corynebacteriumdiphtheriae strain using a sterile inoculation loop. (Inoculation of Test Strain); Inoculate the remainder of the plate with the test strain (unknown) using a sterile inoculation loop. Incubate the plate overnight (16-18 hours) at 37°C . Melt the Tinsdale agar base in a water bath or autoclave. Cool the molten

agar to 57°C . Aseptically add the DifcoTinsdale supplement to the cooled agar base. Mix well to ensure uniform distribution, avoiding bubble formation. Pour the prepared Tinsdale agar mixture into 10 sterile plates. Allow the agar to solidify and dry for 30 minutes to 1 hour. Examine the plates for the presence of a brownish-black coloration around the inoculated areas, indicating cysteinase activity.

Biochemical test(Rosco identification system): Prepare a homogeneous, milky suspension(The milky suspension, also known as a standardized or turbid suspension is a critical component in various microbiological tests, including biochemical identification, antibiotic susceptibility testing, and microbial enumeration) of the test strain in a bijoux containing 2 mL of sterile 0.85% saline solution. To achieve this, gently scrape a small amount of the test strain from an agar plate or broth culture using a sterile inoculation loop. Transfer the inoculum to the bijoux containing saline solution. Adjust the suspension to a standard turbidity, equivalent to 0.5-1.0 McFarland standard (approximately 1-2 x 10^8 CFU/mL). This ensures consistent results. Using a sterile pipette, transfer 0.25 mL of the prepared suspension to each of the five sterile, capped tubes(Arginine,Lysine,Ornithine,Citrulline and Urea). Incubate the tubes at 37°C for 1hr and observe[1,4]

Nitrate reduction diagnostic tablet
Urease diagnostic tablet
Glucose tablet
Maltose tablet
Sucrose tablet

Add one drop of inoculum to a hiss's starch serum water, Inoculate the rosco test at 37°c for 4hours and the hiss starch serum water for 24hours .

RESULTS

TEST	OBSERVATION	RESULTS	INFERENCE
Cysteinase test	Black colonies with black halo	Cysteinase positive	There was no production of enzymes cysteine
Nitrate reduction diagnostic test	Formation of colorless solution	Nitrate reduction negative	Organism in swap does not produce nitrate reductase
Urease diagnostic test	Formation of yellow coloration	Urease negative	Organism in swap did not produce enzyme urease
Glucose test	Formation of red coloration	Glucose test negative	Organism in swap does not produce acid and gas
Maltose test	Formation of red coloration	Maltose test negative	Organism in swap does not produce acid and gas
Sucrose test	Formation of red coloration	Sucrose test negative	Organism in swap does not produce acid and gas
Hiss's starch test	Colorless solution	Negative to hiss starch	There is no break down of starch.

Gram reaction/cell morphology – positive /rod

From the above results, organism is *corynebacterium diphtheria.*

The microscope used to view the organism was brightfield microscope, the Albert's stain was

Fig. 2: Microscopic view of corynebacterium diphtheria after Albert's staining

used for the staining and counter stained with malachite green. This gave a result of rod shape, gram positive and metachromoatic granules(seen as bright green and yellow spots just as the image below). The magnification used was 100*. This was the second series of staining technique used to verify the accuracy.

DISCUSSION/OBSERVATION

Corynebacteriumdiphtheriae is a nonmotile, noncapsulated, club-shaped and Gram-positive bacillus[1]. Corynebacteriumdiphtheriae is classified into biotypes (intermedius, mitis, and gravis) according to colony morphology, as well as into lysotypes based upon corynebacteriophage sensitivity.[1-13] Most strains require nicotinic and pantothenic acids for growth; some also require thiamine, biotin, or pimelic acid [13]. For optimal production of diphtheria toxin, the medium should be supplemented with amino acids and must be.[13] In susceptible individuals, toxigenic strains cause disease by multiplying and secreting diphtheria toxin in either nasopharyngeal or skin lesions. Infection is spread mainly in humans, although toxigenic strains have been isolated from horses. The diphtheritic lesion is often covered by a pseudomembrane composed of fibrin, bacteria, and inflammatory cells.

A PATIENT SUFFERING FROM DIPHTHERIA

Diphtheria toxin can be proteolytically cleaved into two fragments: an N-terminal fragment A (catalytic domain), and fragment B (transmembrane and receptor binding domains). Fragment A catalyzes the NAD+-dependent ADP-ribosylation of elongation factor 2, thereby inhibiting protein synthesis in eukaryotic cells. Fragment B binds to the cell surface receptor and facilitates the delivery of fragment A to the cytosol. Protective immunity involves an antibody response to diphtheria toxin following clinical disease or to diphtheria toxoid (formaldehyde-inactivated toxin) following immunization [13]. In regions where immunization programs are maintained, isolated outbreaks of disease are often associated with a carrier who has recently visited a subtropical region where diphtheria is endemic. Immunization with diphtheria toxoid is extraordinarily effective.[10-12] Diphtheria patients

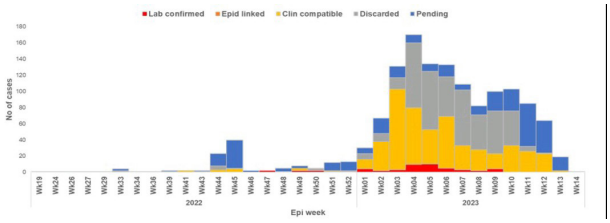

Fig. 3: Epidemic Curve of Diphtheria Cases by Year/ Epi-Week In Nigeria, EPI-WEEK 19 2022 – EPI-WEEK 14 2023

must be promptly treated with antitoxin to neutralize circulating diphtheria toxin.

The outbreak, which started in May 2022, has worsened because of factors such as population growth and climate-related declines in hygiene due to water shortages, and inadequate diphtheria vaccination[12]. These infectious diseases can be controlled by Vaccination. The most effective way to prevent diphtheria is through vaccination, typically given in childhood. Adults who have not had a booster shot in the past 10 years may need one to maintain immunity, good hygiene, Washing hands regularly, especially after contact with an infected person[12]. Avoid close contact with someone who has diphtheria, Proper wound care, Covering and cleaning wounds to prevent bacterial entry. Avoid Sharing utensils, drinks, or personal items with an infected person and most importantly Immunization of contacts, If someone is in close contact with a person who has diphtheria, they may need to be immunized. Proper disposal of contaminated materials and cleaning surfaces will help in the control of diphtheria.

A mangrove plant located at the South-west regio, precisely Ondo state has been found to help in the control of this deadly disease. Volkameriainermis popularly know as ewate by the natives of Ondo has been found to help in retardation of disease growth[1].

The fruits and flowers of these plants are grinded and swallowed and this cause a stagnation of cell growth of any microorganisms found inside the body.[1]

RECOMMENDATION

The future studies of diphtheria (corynebacterium diphtheria) should prioritize

Fig. 4: EWATE(Volkameriainermis)

enhanced surveillance, next-generation vaccine development, and molecular epidemiology. A well Strengthened surveillance systems and improved reporting mechanisms are crucial for accurate tracking of outbreaks and cases. Novel vaccine formulations, such as conjugate vaccines, should be investigated to improve immunogenicity. Genomic analysis of circulating strains and molecular characterization of vaccine escape mutants are essential for monitoring evolutionary changes and tracking transmission patterns. Interdisciplinary research collaborations and global partnerships are vital for accelerating diphtheria research and disease control efforts.

LIMITATION

This study is constrained by several limitations. Firstly, the scarcity of on-site laboratory facilities necessitates reliance on external facilities, potentially introducing logistical challenges. Additionally, the time-intensive nature of diphtheria research requires consistent researcher availability, which can be a hindrance. Furthermore, the paucity of existing literature on diphtheria hampers comprehensive documentation and reporting of research findings, ultimately impacting the study's validity and generalizability. These limitations underscore the need for future studies to address these methodological challenges.

CONCLUSION

Diphtheria is a serious bacterial infection that can have severe consequences, including respiratory failure and death. The prevention of this disease can be said to be general hygiene and reduction in environmental pollution as it places a key role in the wide spread of this disease.

Ethical clearance- NA as the study has no human or animal subjects

Source of funding- None

Conflict of Interest - Nil

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