Haemophilus Influenzae and Haemophilus Parainfluenzae Being the Most Common Secondary Infection Reported Pathogen

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ABSTRACT

INTRODUCTION

Haemophilus influenzae and haemophilus parainfluenzae are considered to be commensal flora of the upper respiratory tract of humans, *belong to family* Pasteurellaceae. The opportunistic *Haemophilus influenzae* is mostly considered as pathogenic while *haemophilus parainfluenzae* have less virulence and rarely cause disease in healthy individuals.Disease caused by either encapsulated (typeable) or unencapsulated (nontypeable) *H.influenzae* .Since the introduction of *H. influenzae* serotype b vaccine, the incidence of infection from typeable type of *haemophilus influenzae* is decreases while nontypeable *hemophilus influenzae* (NTHi) reported for invasive infections. As a secondary infections subsequent respiratory illnesses caused by viruses most commonly involve the lower respiratory tract, with *Haemophilus influenzae* being the most recurrently reported pathogens.

OBJECTIVES

In this study we overview current knowledge on the fast and reliable differentiation method of *haemophilus influenzae* and closely related specie *haemophilus parainfluenzae*. Resistance in *haemophilus influenzae* is also reported due to presence of beta lactamases, a group of enzyme that alters the penicillin binding proteins or the target site of the beta lactam drugs and makes the organism resistant to given beta lactam.

METHEDOLOGY

Total 15 samples of sputum were collected from hospitalized patients. Specimens were belonging to suspected organism *haemophilus influenzae and haemophilus parainfluenzae. Chocolate agar plates (CAP)* were used for inoculation of specimen and for susceptibility testing. *Blood agar plates* and *Mueller Hinton agar plates* were used for identification and differentiation test. Chocolate agar plates (CAP) were used for the specimen inoculation then incubated at 35-37oC for 24- 72hrs in anaerobic environment containing 5-10% CO2. After 24-72hrs of incubation, plates were examined for colonial growth, morphology and characteristics. Examination of appeared colonies on (CAP) were shown round, large, opaque, colorless to grey colonies. No haemolysis or discoloration was seen. Gram stain were also performed and showed gram negative pleomorphic thin rods or coccobacilli under microscope. Confirmatpory test was done using X & V factor followed by kovac's oxidase test. Antimicrobial susceptibility test was fulfilled using CLSI guideline.

RESULTS

These growth requirements are used in the clinical setting to identify suspect organisms to the species level.Commercially available X and V disks were used to analyze the isolates, test were performed on Mueller Hinton agar plates. We were observed colonies that were surrounding the X and XV disc identified as *H.influenzae* and those were surrounding the factor V and factor XV disc identified as *H.parainfluenzae*. Another strategy was made to further confirm the differentiation of both species. Samples were streaked on

blood agar plates which provide heme (X) factor while adding the streaked line of *staph.aerueus* provide NAD (V) factor so in some isolates we observed colonies only satelliting around the streak line of staph.aureus (*H.parainfluenzae*) and in some isolates colonies were appear on whole agar plate it means that, they were utilize both X and V factor that is the characteristic feature of *H.influenzae*.The oxidase test detects the presence of a cytochrome oxidase system .H.influenzae is oxidase positive so upon flooding of kovac's oxidase reagent on selected isolated colonies on CAP, we observed change in color to purple which is positive result for *H.influenzae*.

Susceptibility testing was done by using CLSI susceptibility testing method, performed on CAP. Different antimicrobials were tested against both species such as *Ampicillin (AMP), amoxicillin-clavulanic acid (AMC), ceftriaxone (CRO), trimethoprim-sulfamethoxazole (SXT), ciprofloxacin (CIP), ofloxacin (OFX) and chloramphenicol (C).* Our result appeared as *H.parainfluenzae* showed such sensitivity pattern AMP75%, AMC83%, CRO83%, SXT42% , C100% , CIP/OFX75% while 100% sensitivity reported by *H.influenzae* towards AMC, SXT, AMP, CRO, and 66% sensitivity against CIP/OFX and C .

CONCLUSION

The objective of my study project was to conventional identification of *H.influenzae* and *H.parainfluenzae*, which was achieved by using different methods of differentiation. In this pandemic situation due to covid-19 virus, the secondary infection as well as opportunistic infections have been increased. So we should take a keen interest to identification of other causative agent including bacteria and to evaluate the antimicrobial susceptibility of these organisms to overcome the seveirity of infections. My results showed the some of the resistivity against the different antimicrobial drugs. Due to emergence of resistance it's needed to pay more attention to control the resistance to these antibiotics as well as emergence and spread of resistance to other antibiotics, such as new fluoroquinolones, new beta-lactams, and new macrolides**.**

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