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بسم اللّه الرحمن الرحيم

مركز الشبكات وتكنولوجيا المعلومات

قسم التوثيق الإلكتروني

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم باللّه العظيم أن المادة التي تم توثيقها وتسجيلها

علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

Determination of Antibiotic Resistance Pattern of *Haemophilus Influenzae* isolated from Egyptian patients in Ain Shams University Hospitals

Thesis

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Radwa Shereen

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List of Abbreviations

Abb.	Full-Term
CAP	Community-Acquired Pneumonia
CF	Cystic Fibrosis
CLSI	Clinical and Laboratory Standards Institute
COPD	Chronic Obstructive Pulmonary Disease
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
Hib	*H. influenza* type b
HMW	High Molecular Weight
HTM	Haemophilus Test Media
IV	Intravenously
LPD	Lipoprotein D
NP	Nasopharyngeal
NTHi	Non-Typeable *H. influenza*
OM	Otitis Media
OMP P2	Outer Membrane Protein P2
OMVs	Outer Membrane Vesicles
PBP3	Penicillin Binding Protein 3
PCR	Polymerase Chain Reaction
PLPAR	β-lactamase-Positive ampicillin-Resistant
PRP	Polyribosyl-Ribitol-Phosphate
QRDRs	Quinolone-Resistance Determining Regions
RNA	Ribonucleic Acid
SIADH	Syndrome of Inappropriate Antidiuretic Hormone Secretion
UV	Ultraviolet
WHO	World Health Organization

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INTRODUCTION

Haemophilus influenzae is a small (1 μm × 0.3 μm), pleomorphic, gram negative coccobacillus. Some strains of *H influenzae* possess a polysaccharide capsule, and these strains are serotyped into 6 different types (a-f) based on their biochemically different capsules. The most virulent strain is *H. influenzae* type b (Hib). Some *H. influenzae* strains have no capsule and are termed nonencapsulated *H. influenzae* or nontypeable *H. influenzae* (NTHi) ***(Tang et al., 2015).***

Haemophilus influenzae is recognized as a frequent cause of a variety of infections among outpatients, including acute otitis media, sinusitis, acute purulent exacerbation of chronic bronchitis, and pneumonia. These infections are usually caused by non-type b strains of *H. influenzae*. *Haemophilus influenza* type b (Hib) is responsible for severe pneumonia, meningitis and other invasive diseases almost exclusively in children aged less than 5 years. Hib also causes potentially severe inflammatory infections of the mouth, epiglottis, joints, bones, peritoneum, and trachea. Between 3% to 6% of Hib cases in children are fatal; up to 20% of patients who survive Hib meningitis have permanent hearing loss or other long-term neurological sequelae. Patients ≥ 65 years of age with invasive *H. influenzae* disease (Hib, non-b, and nontypeable) have higher case-fatality ratios than children and young adults ***(Kotirum et al., 2015).***

The resistance of *H. influenzae* to commonly used

antimicrobials has risen in recent years, and wide variations exist in antimicrobial susceptibility rates, both geographically and over time. Ampicillin resistance in *H. influenzae* is now globally widespread, with incidence rates varying from 8 to 30 % in different European countries and North America to more than 50 % in some Eastern Asian countries, with increasing resistance to ampicillin, amoxicillin, amoxicillin-clavulanate, and many cephalosporins, limiting the efficacy of expanded-spectrum cephalosporins in cases of meningitis and of many oral cephalosporins in other diseases. Most strains remain susceptible to the carbapenems,and quinolones ***(Resman et al., 2012).***

Two β-lactam resistance mechanisms have been described. One involves enzymatic hydrolysis of β-lactam by TEM-1 or ROB-1 β-lactamases, and such isolates are denoted β-lactamase-positive ampicillin-resistant (BLPAR). The other involves decreased β-lactam affinity for penicillin binding protein 3 (PBP3) owing to alteration in the ftsI gene ***(Maddi et al., 2017).***

AIM OF THE WORK

The aim of the present thesis is to identify the *Haemophilus* species isolated from clinical samples of patients referred to the Main Microbiology Laboratory, Ain Shams University Hospitals & determine its local resistance pattern.

REVIEW OF THE LITERATURE

Taxonomy of *Haemophilus influenza*

Kingdom: Bacteria, Phylum: Proteobacteria, Class: Gammaproteobacteria, Order: Pasteurellales, Family: Pasteurellaceae, Genus: *Haemophilus* ***(Lansink et al., 2017).***

The genus *Haemophilus* comprises eight bacterial species, which are associated with humans (*Haemophilus influenzae*, *H. aegyptius*, *H. ducreyi*, *H.pittmanniae*, *H.parainfluenzae*, *H.haemolyticus*, *H.parahaemolyticus*, and *H. paraprohaemolyticus*). They are small, nonmotile, pleomorphic gram-negative bacteria and the morphology ranges from small coccobacillary forms to short rods. All species are facultative anaerobes ***(Lansink et al., 2017).***

Haemophilus (meaning blood-loving) refers to the specific dependence of this organism on heme-related molecules for growth under aerobic conditions. These are X (hemin) and V (nicotinamide-adenine-dinucleotide). These factors are released following lysis of red blood cells, thereby allowing growth of this fastidious organism on chocolate agar. X-factor is protoporphyrin IX,a metabolite in haemin biosynthesis. V-factor is a coenzyme composed of nicotinamide compounds (nicotinamide adenine dinucleotide & nicotinamide adenine dinucleotide phosphate) ***(Aubrey et al, 2020).***

Classification

Haemophilus influenzae is a Gram-negative bacterium that is classified by the presence or absence of a polysaccharide capsule, termed "typeable" and "non-typeable" *H. influenzae* (NTHi), respectively ***(Whittaker et al,2017).***

Typeable *H. influenzae*

Encapsulated strains (also known as typeable) are surrounded by a polysaccharide capsule that plays an important role in the determination of virulence of the organism. The outer membrane lipo-oligosaccharides also contribute to the degree of virulence. there are 6 encapsulated serotypes (*H. influenzae* serotypes a [Hia], b [Hib], c [Hic], d [Hid], e [Hie], and f [Hif]) ***(Whittaker et al., 2017).***

H. influenzae type b is a significant cause of invasive bacterial infections and can occasionally cause fatal invasive infections, such as meningitis and acute epiglottitis. Since the Hib vaccine was first introduced and has been used for more than 10 years in Western countries, non-type b *H. influenzae* infections are expected to be the more prevalent type of *H. influenzae* infections worldwide ***(Hasegawa et al., 2020).***

Non-typeable *H. influenzae*

Un-encapsulated strains are termed non-typeable *H. influenzae* (NTHi). NTHi induces a polymicrobial disease typically due to predisposing upper respiratory tract viral infection. According to recent reports, one of the most common causes of invasive infections by *H. influenzae* in older adults was Hif, following NTHi in Western countries ***(Hasegawa et al., 2020).***

Genome Structure

Haemophilus influenzae is a genetically divergent species with the core-genome consisting of 1308 genes present in all strains, and 2116 compose the accessory gene. The obtained accessory genome was larger than any single genome (mean of 1767 coding sequences per genome), suggesting that *H. influenzae* could have an open pangenome, due to its competence in acquiring genomic material throughout the infection process ***(Pinto et al., 2018).***

Non Typeable *Haemophilus influenzae* possess a remarkable ability to acquire novel genomic material during infection and it has been predicted that the *H. influenzae* pan-genome could reach up to ~6000 distinct genes, with isolates acquiring genetic material during the co-colonization of the respiratory tract with other opportunistic bacteria, such as *Streptococcus pneumoniae* or *Moraxella catarrhalis*. While