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MOLECULAR DETECTION OF VIRULENCE GENES OF *Pseudomonas aeruginosa*

CAUSING AN OUTBREAK IN A TERTIARY CENTRE, SELANGOR.

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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen which is commonly associated with healthcare associated infection (HAI) and outbreaks. They have the ability to possess multiple pathogenic factors which play an important role in causing invasive infections such as surgical site infection (SSI), osteomyelitis, pneumonia, and blood stream infection. Between 2016 to 2017, there were two outbreaks caused by sensitive strain *P. aeruginosa* in a Tertiary Centre among 17 patients which resulted in multiple complications and fatalities. The outbreak investigation conducted using pulsed field gel electrophoresis (PFGE) revealed seven clonally related *P. aeruginosa* strains from A to G. The objective of this study is to determine the virulence factors acquired by *P. aeruginosa* isolates obtained from the clinical samples during the outbreak and to describe the clinical outcome associated with these infections. The *P. aeruginosa* isolates from outbreak investigation were retrieved from the laboratory's stock culture collection and revived to determine their virulence genes by polymerase chain reaction (PCR). Six virulence genes were investigated, namely *ToxA*, *ExoS*, *LasI*, *LasB*, *OprI*, and *OprL* genes which encodes for exotoxin A, exoenzyme S, quorum sensing system, alginate, and the last two genes are for peptidoglycan related outer membrane respectively. A total of ten out of 17 clinical isolates, were able to revive. They were represented by clone A, B, C, D, F and G. Results showed that *ToxA* gene were detected in six isolates only which belonged to clone A (one isolate) and clone C (five isolates). While *ExoS*, *LasI*, *LasB*, *OprI*, and *OprL* were detected in all six clones. The *P. aeruginosa* isolate which belonged to clone A caused pneumonia while *P. aeruginosa* which belonged to clone C caused surgical site infections which also resulted in complication such as disseminated infections and death. The preservation and the presence of multiple virulence genes among these *P. aeruginosa* isolates were possible contributing factors to the invasiveness, persistence, and severity of the infection despite the organism being sensitive to the antimicrobials tested. Further investigations of *P. aeruginosa* virulence genes involving a larger number of isolates and involving different types of infection may reveal more variable virulence genes pattern and would provide a better correlation with their clinical outcomes.

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CHAPTER ONE
INTRODUCTION

1.1. Research Background

Pseudomonas aeruginosa is the major human pathogen among the *Pseudomonas* species. It is an aerobic gram-negative, rod-shaped bacteria that is motile and does not ferment lactose [1].

P. aeruginosa is an opportunistic organism which causes infection in patients with underlying co-morbidities such as burns, malignancies, human immunodeficiency virus (HIV) infection, surgical site infections (SSI), patients with catheter in situ, post-solid organ transplant [2] as well as in cystic fibrosis patients [3]. In 2017, the World Health Organization (WHO) published a list of antibiotic resistant “priority pathogens” which have the potential to be a threat to human health which includes *P. aeruginosa* [4]. This is due to the ability of *P. aeruginosa* to develop resistance to treatment and to transfer its resistance genes to other bacteria.

P. aeruginosa is also a common causative agent of healthcare associated infection (HAI). A systematic literature review and meta-analysis by Ling et. al. 2015 on the burden of HAI in South-East Asia showed that the prevalence of overall HAI is 9.0%, and the estimated incidence of surgical site infections was 8.6% with *P. aeruginosa* being one of the most common microorganisms identified for overall HAIs [5], among *Klebsiella spp.* and *Acinetobacter baumannii. P. aeruginosa* accounts for 11% of all nosocomial infection causing surgical and wound infections, urinary tract infection, pneumonia and also bacteraemia [6].

P. aeruginosa contains numerous virulence factors which commonly encountered in other bacteria. They form biofilm, exotoxin, pili, flagella and through quorum sensing system making them resistant to multiple antibiotics which have contributed to the infections in vulnerable hospitalized patients [7]. Many studies have shown that different virulence factors of *P. aeruginosa* contribute to different type of infections such as acute invasive or persistent infections [3], [8]. With the increasing number of multidrug resistant strain of *P. aeruginosa*, many treatment modalities have been directed towards targeting the virulence factors [2]. Many hospital outbreaks of *P. aeruginosa* are linked