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Case Report

The Prevalence of Biofilm Formers Among Clinical Isolates of *Klebsiella pneumoniae*: A Meta-Analysis

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Abstract

Background: The study explored the prevalence of biofilm formers in *Klebsiella Pneumonia*, a gram-negative bacterium that has high propensity to form antibiotic resistant strains and forms biofilms. Biofilms are complex microbial community with attributes that vary from planktonic cells.

Methods: A single-armed meta-analysis was done to assess prevalence of biofilm formers. Published studies were reviewed from PubMed and Scopus. A random effects meta-analysis was done. Freeman-Tuckey double arcsine method was selected for transformation. Publication bias was assessed using Doi plot and Luis Furuya-Kanamori (LFK) index.

Results: 23 studies were selected for the review. The meta-analysis revealed 74% (95% CI: 64%-83%) prevalence of biofilm formers among clinical isolates of *Klebsiella pneumonia*. The indices of heterogeneity among the included studies was high, indicated by a p value <0.01 and I^2 = 94.4%. Doi plot showed asymmetry marked by unequal deviation and lengths of the arms. The LFK index of -0.67.

Conclusions: The prevalence rate is comparable with that of prevalence rate attained by other bacterium by similar meta-analysis studies. This high prevalence of biofilm formers warrants for a paradigm shift in treatment strategies for treatment of infections.

Keywords: Biofilm; *K. Pneumonia*; Meta-analysis

Abbreviations

BF: Biofilm; CDC: Centers for Disease Control and Prevention; CI: Confidence Interval; EPS: Extra Cellular Polymeric Substances; LFK: Luis Furuya-Kanamori; MDR: Multidrug Resistance

Introduction

Biofilms are formed by a community of microorganisms that adhere to a living or non-living surface thereby forming a matrix bound by polysaccharides or extra cellular polymeric substances (EPS) [1]. Bacterial biofilms can be formed from one or multiple bacterial species [2]. Biotic and abiotic surfaces like tissues, medical devices and other surfaces can be home to biofilms [3-5]. Gene expression vary between biofilms and planktonic cells that enhance, among other traits, resistance to antibiotics [6]. Bacterial biofilms form an effective defense mechanism for the microorganisms against antibiotics and the immune cells.

The claims of tolerance to antibiotics and host immunity by biofilms has led it to be considered potent threat to public health. The means for protection of microorganisms in biofilms are distinct from isolated planktonic microorganisms. It has been hypothesized that biofilms hinder the penetration of antibiotics, slow growth rates, induce adaptive stress response and bring about expression of specific genetic determinants of antibiotic resistance [7,8]. The EPS matrix serves as a protective layer that acts as a physical barrier or binds with the antibiotic.

Detection of BF formation is conducted mainly by three methods, namely tissue culture plate method [9], tube method [10] and Congo red agar method [11]. Visual assessment of biofilms relies on microscopy. Electron microscopes were initially used for the examination and later on to more advanced techniques of microscopy like confocal laser scanning microscopy (CLSM) [12].

K. pneumoniae's ability to produce biofilms was explained by LeChevallier., *et al*. in 1988 in his experiment to understand the factors promoting survival of bacteria in chlorinated water supplies [13].

Biofilms by *K. pneumoniae* have been demonstrated to be isolated from various body samples like urine, wound swabs and blood in multiple studies. Type 1 and 3 fimbrae and capsules are structural phenotypes that have been demonstrated to play vital roles in biofilm formation of *K. pneumoniae* [14]. It has also been observed that the genes that code for fimbrae and capsule along with a wide range of genes including those that code for transcriptional regulators, sugar phosphotransferase homologues, cellobiose (celB) and quorum sensing (luxS) and genetic loci of unknown function affect the formation of biofilm in *K. pneumoniae* [15-17].

The objective of the study is to estimate the global prevalence of biofilm formers in clinically isolated *K. pneumoniae.* The goal of the proposed study is to guide therapeutic approaches for *K. pneumoniae* infections.

Methods

Eligibility criteria

The inclusion criteria included all studies that were published since 2012 that presented prevalence of biofilm formers in of *K. pneumoniae.* Studies that were conducted using clinical isolates from human were only selected. Only studies that used Tissue Culture Plate method or Congo Red Agar method for detection of biofilm formation was included. Studies that presented prevalence based on specific subsets like Carbapenem-resistant *Klebsiella pneumoniae* or extended-spectrum β-lactamases (ESBL) producing *K. pneumoniae* and studies in languages other than English were excluded.

Search strategy

Electronic bibliographic databases, PubMed and Scopus, were used for searching for published studies. The key words from the research question, *K. pneumoniae* and biofilm formation, were used to identify appropriate search terms. The search terms used in PubMed and Scopus is given in Appendix. The studies that returned from the search were initially filtered by reviewing the titles and abstracts and the language used, followed by review of the full text. Information required was extracted in a form, in which the population samples, the prevalence estimate, biofilm detection method and other information was extracted.

Analysis

A random effects meta-analysis was done. The combined prevalence of BF formation among the isolates was determined with 95% confidence interval (CI) and Forest plot was generated. Proportion values were used to present the results (0 to 1). In the case of meta-analysis of proportions, in order to overcome the normal distribution assumption by conventional meta-analysis technique and to ensure variance stabilization the individual proportions are transformed. Freeman-Tuckey double arcsine method was selected for transformation. Heterogeneity was assessed using I2 statistic. Publication bias was assessed using Doi plot and Luis Furuya-Kanamori (LFK) index. The codes used in R for the meta-analysis is given in Appendix.

Result

956 and 1820 studies were identified from PubMed and SCOPUS, respectively. After the removal of 674 duplicates and 1967 studies that were either reviews or not in English or did not have a matching title or abstract as per the eligibility criteria, there remained 135 studies. Full texts of these studies were reviewed and 23 studies were selected for the review. Out of these studies, the highest number were from India (7 studies) followed by studies from Iran (6 studies), Egypt (2 studies), Pakistan (2 studies), Spain (2 studies), Ethiopia (1 study), Indonesia (1 study), Nepal (1 study) and Mexico (1 study). The sample sizes of the studies ranged from 200 to 8 with a mean sample size of 74.

The mean overall prevalence of BF formation among clinical isolates of *Klebsiella Pneumoniae* ranged from 0.97 to 0.18. Both, the highest prevalence and lowest prevalence was seen in studies

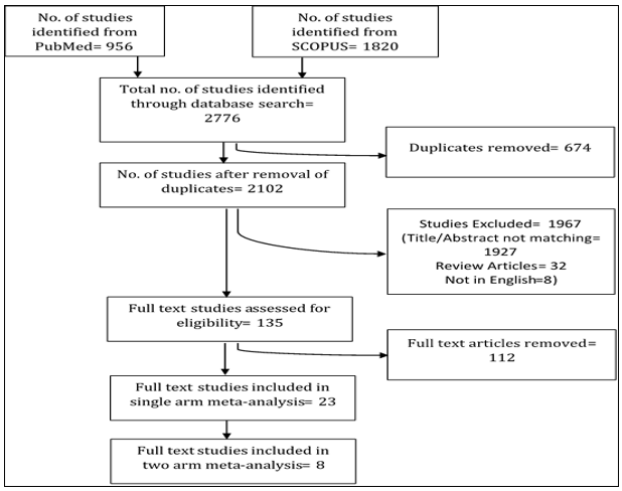

Figure 1: Selection of studies.

that were conducted in India. Combined data from 23 studies were pooled to assess the prevalence of BF formation among clinical isolates of *Klebsiella pneumoniae*. The combined prevalence was calculated as 0.74 (95% CI: 0.64-0.83) and 0.75 (95% CI: 0.73-0.77), respectively in the random effects model and common effect model. The indices of heterogeneity among the included studies was high, indicated by a p value < 0.01 and I^2 = 94.4%. Doi plot showed asymmetry marked by unequal deviation and lengths of the arms. The LFK index of -0.67.

Figure 2: Forest plot of the meta-analysis of prevalence of BF formation among clinical isolates of *Klebsiella Pneumoniae*.

Figure 3: Doi plot of the meta-analysis of prevalence of BF formation among clinical isolates of *Klebsiella Pneumoniae*.

Discussion

The pooled prevalence of BF formation in clinical isolates of *K. pneumoniae* was 0.74 (95% CI: 0.64-0.83). The pooled prevalence was synthesized from the 23 studies, comprising of 1713 clinical isolates of *K. pneumoniae.* Out of the 23 studies that were selected for this review, 10 studies had a prevalence rate lesser than the pooled prevalence rate and 13 studies had prevalence rate that is higher than the pooled prevalence rate. The studies that were reviewed here had varied methods for detection of BF formation, which consisted of tissue culture plate method [10], tube method [11] and congo red agar method [12] as given in Appendix. Although the tissue culture plate method is considered to be the gold standard for detection of BFs, the study has included other methods like tube method and congo red agar method as they are comparable [18].

Several studies have been conducted to assess the BF formation in clinical isolates of bacteria. A similar meta-analysis study that evaluated the prevalence of BF producers among clinical isolates of *Escherichia coli* found a similar prevalence of 74.4% [19]. Meta-analyses that assessed the prevalence of BF formation in clinical isolates of Pseudomonas aeruginosa, a gram-negative bacterium, showed a prevalence of 87.6% and 86.5%, globally and among the Iranian population respectively [20-22]. A study conducted by Sanchez., *et al*. that included both gram positive and gram-negative clinical isolates showed a comparable BF formation rate of 61.4% [6].

Author	Year	Location	BF determination method
Ahmed., *et al*.	2022	Egypt	Microtiter-Plate Method
Alcántar-Curiel., *et al*.	2018	Mexico	Microtiter-Plate Method
Asati and Chaudhary	2017	India	Modified Tissue Culture Plate
Ashwath., *et al*.	2022	India	Microtiter-Plate Method
Ballén., *et al*.	2021	Spain	Microtiter-Plate Method
Bobbadi., *et al*.	2021	India	Microtiter-Plate Method
Cepas., *et al*.	2019	Spain	Microtiter-Plate Method
Maiti., *et al*.	2014	India	Microtiter-Plate Method
Dumaru., *et al*.	2019	India	Tube adherence and Congo red agar
Eghbalpoor., *et al*.	2019	Iran	Microtiter-Plate Method
El-Domany., *et al*.	2021	Egypt	Tube method
Ghanizadeh., *et al*.	2021	Iran	Microtiter-Plate Method
Imtiaz., *et al*.	2021	Pakistan	Microtiter-Plate Method
Karimi., *et al*.	2021	Iran	Microtiter-Plate Method
Kodori., *et al*.	2021	Iran	Microtiter-Plate Method
Mirzaie and Ranjbar	2021	Iran	Congo red agar test
Mishra., *et al*.	2015		Microtiter-Plate Method
Nirwati., *et al*.	2019	Indonesia	Microtiter-Plate Method
Kuwa., *et al*.	2021	Ethiopia	Microtiter-Plate Method
Rahim., *et al*.	2016	Pakistan	Congo red agar test
Shadkam., *et al*.	2021	Iran	Microtiter-Plate Method
P. Subramanian	2012	Pondicherry	Microtiter-Plate method

Appendix 1: BF detection method and location of studies.

Conclusion

This review's finding that among clinical isolates of *K. pneumoniae* there was a prevalence of 74% of BF forming isolates indicates the ubiquitous nature of BF amongst the species. Comparable prevalence has been found in other species of bacteria also. This asserts the importance of research to shift paradigms to focus BF counterparts of planktonic bacteria for developing effective clinical alternatives. Tangible evidence to understand the mechanism of antibiotic resistance would be required to nudge further research.

Ethical Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of data and Materials

The data that support the findings of this study were derived from the following resources available in the public domain.

Database: URL

PubMed: https://pubmed.ncbi.nlm.nih.gov/

SCOPUS: https://www.scopus.com/

Competing Interest

I declare that I have no conflicts of interest.

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Authors' Contributions

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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