

Molecular Basis of Biofilm Production and Antibiotic Resistance in Klebsiella Species from Diabetic Ulcer

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Abstract: ***Aim:** The aim of this study is to investigate molecular basis of biofilm production and antibiotic resistance in Klebsiella species from diabetic ulcer. Diabetes mellitus is a chronic metabolic condition characterized by high blood glucose, often known as blood sugar. Infected wounds are a common complication among diabetics. **Design and Method:** A systematic literature review to identify peer-reviewed studies on molecular basis of biofilm formation and antibiotic resistance in Klebsiella species from diabetic ulcers. The inclusion criteria for the search included finding an English language article published in a peer reviewed journal that included our keywords and discussed the antibiotic resistance and molecular basis of biofilm formation of Klebsiella species from diabetic ulcer, including early works pertinent to current review. According to data published by the World Health Organization, the prevalence of diabetes mellitus has gradually increased over time. Diabetic foot ulcers are estimated to impact approximately 10%-15% of those with diabetes at some point. Bacterial strains obtained from the wounds of individuals with diabetes exhibit various types, encompassing gram-positive and gram-negative bacteria. Multidrug resistant organisms are frequent in diabetic foot ulcer patients. Biofilms may impair antibiotic penetration into the wound bed or render leukocytes ineffective by establishing antiphagocytic characteristics inside the biofilm matrix. Klebsiella species has also been identified as a major isolate in diabetic ulcers in several investigations. The goal of this study is to look at molecular characteristics of biofilm development and antibiotic resistance of Klebsiella in diabetic ulcers.*

Keywords: Diabetic ulcer, Biofilm, Antibiotic resistance, Klebsiella species, Polymerase Chain Reaction

Abbreviations

Ckp - Classical Klebsiella pneumoniae
CONS - Coagulase Negative Staphylococcus aureus
ESBL - Extended Spectrum Beta Lactamase
Hvvp - Hypervirulent Klebsiella pneumoniae
Hmvkp - Hypermucoviscous Klebsiella pneumoniae
K.pneumoniae - Klebsiella pneumoniae
MRSA - Methicillin Resistant Staphylococcus aureus
P.aeruginosa - Pseudomonas aeruginosa
PCR - Polymerase Chain Reaction
VRE -Vancomycin Resistant Enterococcus

1. Introduction

Diabetes mellitus (DM), a metabolic condition, is spreading at an alarming rate over the world. Today, India has over 33 million diabetics. It is the country with the most diabetes and is known as the "diabetic capital of the world."¹The number of diabetes in India is anticipated to reach 79.4 million by 2030.²Diabetic patients are more prone to infections due to elevated glucose levels, impaired immunological responses, and decreased blood supply to extremities, which results in sluggish healing wounds.³

Diabetes foot infections produced by various bacteria genera are the most dreaded consequences of diabetes, with substantial morbidity and the possibility of gangrene and amputation. Diabetes foot infection is common.⁴ Wound infection is one of the leading causes of health-care problems because the ensuing wound is colonized by

dangerous microorganisms. In hospitalized patients, infection is a major cause of morbidity and mortality. Gram positive bacteria, which were once the dominating organisms in wound infection, have recently been supplanted by gram negative bacteria. Among gram negative bacteria, Klebsiella species are on the rise in wound infection.⁵Because the bacteria that cause diabetic foot infection have developed resistance to a number of current medications, the most effective infection management strategy is regular debridement of foot ulcers.⁶

Klebsiella is a Gram-negative, encapsulated, nonmotile, rod-shaped bacterium that belongs to the Enterobacteriaceae family and can cause gastrointestinal, skin, nasopharyngeal, osteomyelitis, biliary and urinary tract infections, and bacteremia.⁷ Klebsiella pneumoniae strains are more frequent in immunocompromised people such as diabetics, the elderly, and children, and they are extensively colonized in hospitalized patients.⁸

The misuse of antibiotics has complicated the treatment of Klebsiella pneumoniae and limited our alternatives for efficient therapy of this bacterial illness.⁹Previous research has revealed that K. pneumoniae strains resistant to a wide range of antibiotics are rapidly spreading, particularly when the bacteria are capable of building biofilm.¹⁰These bacteria may produce a thick coating of extracellular biofilm, which aids in their attachment to living and abiotic

surfaces.¹¹Infections produced by biofilm-forming strains are more difficult to treat than other strains.¹²

Numerous genes, including both fimbrial and non-fimbrial adhesion genes like the *mrkD* gene, are involved in the virulence of *K. pneumoniae*. The *mrkD* gene is thought to act as the type 3 fimbrial adhesin in the biofilm of *K. pneumoniae* and is a significant virulence factor that increases the capacity to cause chronic infection.¹³

Antibiotic resistance and bacterial biofilm development may be important factors in the establishment of multi drug resistant *Klebsiella pneumoniae* strains.¹⁴Because of the antiphagocytic nature of biofilm, it is more difficult for host defense to eradicate this type of bacterial infection.¹⁵The use of antibiotics in individuals with bacterial illnesses can result in bacterial eradication and speed up the therapeutic process. Consequently, the rising prevalence of drug resistance leads to patient difficulties and greater medical expenses.¹⁶By overcoming some of the drawbacks of traditional biochemical and serological methods, molecular methods for bacterial diagnosis have demonstrated benefits in terms of increased sensitivity and rapidity.¹⁷

2. Methodology

The systematic review study is carried out utilizing online databases and pertinent research publications related to the molecular basis of biofilm formation and antibiotic resistance of *Klebsiella* in diabetic ulcers, published from 2004 up to 2023. Scholarly publications were retrieved using keywords primarily from several research database sites such as Google Scholar, PubMed, and Scopus. If the case studies and clinical reports related to our interest theme, they were included.

3. Review of Literature

Dr. S Chavan et al. conducted a research to analyze the clinical and bacteriological profile of diabetic foot ulcers among rural Indian residents, and to know antimicrobial resistance of most often isolated organisms to various antimicrobial drugs. In their study, a total of 78 specimens were cultured and 97.4% ulcer showed growth of organisms, both aerobes and anaerobes. Only 5 of the 139 isolates were anaerobes, whereas 134 were aerobes. The most common organism was *Pseudomonas aeruginosa* (23.9%), followed by *Klebsiella* species (20.9%). 30.6% aerobic gram positive bacteria were isolated. *Staphylococcus aureus* was the most common gram positive organism (28.4%) in which Methicillin resistant *S. aureus* (MRSA) was 57.9%. Among the gram negative organisms, 46 isolates showed ESBL production. Among ESBL producing strains, *Escherichia coli* (68.3%) isolates recorded highest ESBL activity followed by *Pseudomonas aeruginosa* (62.5 %) and *Klebsiella* species (53.6 %).¹⁸

Asmaa M Elbrolosy conducted a study to evaluate the frequency and pattern of antibiotic resistance of clinical *K. pneumoniae* isolates at Menoufia University Hospitals and to gain insight into the function of the *mrkD* gene as a regulator of biofilms. Hospitalized and outpatient clinic patients provided a total of 340 unique clinical samples.

With the use of the vitek-2 system and conventional microbiological techniques, 84 *K. pneumoniae* isolates were found. Using the disc diffusion method, the pattern of antimicrobial resistance was identified. Modified Congo Red agar method was used to phenotypically demonstrate the biofilm formation of *K. pneumoniae*. The presence of *mrkD* gene as a genetic determinant of biofilm formation was confirmed by PCR. The most frequently isolated organism was *Klebsiella* species followed by *E. coli*, *Staph. aureus*, *Pseudomonas*, *Enterobacter sp.*, *Acinetobacter sp.*, and *Proteus sp.* *Candida sp.* *K. pneumoniae* was the most common *Klebsiella* species identified by the Vitek-2 method, followed by *K. oxytoca*, and *K. ozaenae*.¹⁹

Shimaa A. Abdel Salam et. al. conducted a study in which 80 isolates of *K. pneumoniae* from the main microbiology laboratory of Ain Shams University hospital were tested for antibiotic susceptibility, biofilm formation ability, phenotypic identification and *rmpA* gene detection by PCR. The majority of the isolates were detected in sputum samples than urine and pus samples. 46.25% isolates were identified phenotypically as *hmvpk* while 53.75% were classic *K. pneumoniae*. Most of the *hmvpk* isolates were biofilm producers. *cKp* made up the majority of the isolates. Both exhibit strong resistance to cephalosporin and beta lactam antibiotics, yet greater susceptibility to meropenem and imipenem. All of the *K. pneumoniae* isolates showed the distribution of the *rmpA* gene.²⁰

Another epidemiological study in Northeastern Italy by Giovanni Boschetti et.al showed that bacterial growth was detected in 406 samples of diabetic foot ulcer. The bacteria isolated were mainly resistant to one or more than two classes of antibiotics. Only 15.7% of the *Staphylococcus aureus* strains were sensitive to all antibiotic classes, while the *Escherichia coli* and *Pseudomonas aeruginosa* strains displayed an absence of resistance in only 18.75% and 33.8% of samples, respectively. In two cases, *Klebsiella pneumoniae* bacteria were resistant to carbapenems, with twofold resistance to fluoroquinolones but not to cotrimoxazole. *Pseudomonas aeruginosa* strains had a different pattern of double resistance.

Staphylococcus aureus and *Pseudomonas aeruginosa* accounted for approximately half of the cases and exhibited extensive antibiotic resistance. MRSA was found in 52 instances, whereas the remaining 27 samples (14.06%) demonstrated resistance to three separate antibiotic classes but no methicillin resistance. Only isolated *Enterococcus faecium* (VRE pattern) demonstrated resistance to vancomycin, teicoplanin, gentamicin, kanamycin, ampicillin, and imipenem, whereas all *Morganella morganii* were resistant to at least three distinct classes of antibiotics. Lastly, *Staphylococcus aureus* strains, resistant to oxacillin (MRSA), were often refractory, even against fluoroquinolones (52/45) and MLSB (macrolide, lincosamide, streptogramin B: 52/29), while much less so to aminoglycosides (52/19) and tetracycline (52/9).²¹

Another Research conducted by Amir and Reza to determine antibiotic resistance, virulence-associated genes analysis, as well as molecular typing of *K. pneumoniae* strains. From 505 clinical samples taken from hospitalized patients, 100 *K.*

pneumoniae strains were isolated using conventional microbiological methods and evaluated phenotypically and genotypically. Among 100 *K. pneumoniae* strains, 75% were resistant to ciprofloxacin, 73% to trimethoprim-sulfamethoxazole, and 68% to nitrofurantoin whereas 28% and 52% strains were responsive to streptomycin and imipenem. 77% of *K. pneumoniae* strains were able to form biofilm and 89% of biofilm formers were multi drug resistant. *fimH*, *mrkA* and *mrkD* were detected in all types of urine, blood and CSF isolates. The enterobactin biosynthesis gene (*entB*), serum resistance associated outer membrane lipoprotein (*traT*) and regulators of mucoid phenotype A (*rmpA*) were detected in 80%, 62% and 48% of *K. pneumoniae* strains. In their investigation, identified 11 patterns in 100 *K. pneumoniae* strains using repetitive element sequence based-PCR, with the majority of MDR strains falling into the same pattern.²²

Another study by Ayesha Nageen was done to find out the most prevalent organism in diabetic foot ulcers and its drug sensitivity and resistance to different standard antibiotics. In their study, ninety-five diabetic patients with infected foot wounds who had not received any previous antibiotics were included by consecutive sampling. Pus culture specimens were obtained from wounds and *Staphylococcus aureus* was the most common organism identified, accounting for 23.16% of all isolates, followed by *Escherichia coli* and *Klebsiella*. According to their findings, *Staphylococcus aureus* was the most prevalent pathogen among all patients, independent of gender, age group, or co-morbidity, and Meropenem was the most effective antibiotic while cotrimoxazole was the least effective.²³

Priyadarshini Shanmugam et al. concluded in their research that Gram negative bacilli were more abundant (65.1%) than Gram positive cocci (34.9%). For their investigation, they collected 75 bacterial isolates from 50 diabetic foot ulcer patients. The most prevalent isolate was *Pseudomonas* species, followed by *Escherichia coli* and *Staphylococcus aureus*. *Streptococcus pyogenes*, *Klebsiella* species, *Acinetobacter* species, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Proteus mirabilis*, *Citrobacter* species and *Enterococcus* species, CONS, and *Enterobacter* species were also isolated. The antibiotic sensitivity profiles of the bacteria were also studied. 37.5% of the gram negative bacilli were ESBL producers and 31% were carbapenemase producers.²⁴

In another research by Mohammed Talal Alharbi et.al to determine the frequency of hyper virulent *K. pneumoniae* among hospital-acquired *K. pneumoniae* infections in the intensive care unit (ICU) and to compare hypervirulent *K. pneumoniae* and classical *K. pneumoniae* in terms of antibiotic resistance pattern, pathogenicity, and molecular characteristics. In their study, *K. pneumoniae* isolates were subjected to antimicrobial susceptibility testing and detection of extended-spectrum- β -lactamase (ESBL) production by the Phoenix 100 automated microbiology system, string test, biofilm formation, serum resistance assay, and detection of virulence-associated genes (*rmpA*, *rmpA2*, *magA*, *iucA*) and capsular serotype-specific genes (K1, K2, K5, K20, K57) by polymerase chain reaction (PCR). 120 ICU patients suffering from hospital acquired

K. pneumoniae infections were included. The hmv phenotype (based on the string test) was detected in 22.5% of all *K. pneumoniae* strains. This phenotype was much greater in hvKp strains. Fifty-three strains were identified as ESBL-producing strains, which were frequent in the classical *K. pneumoniae*. Their research found that the hvKp isolates were highly associated with moderate and strong biofilm formation and they have significantly higher capsular serotype specific genes K1 and K2. Hmv phenotype *rmpA*, *rmpA2* and *magA* were significantly associated with hypervirulent *K. pneumoniae* strains.²⁵

A study by Jean-Marie Liesse Iyamba et.al showed that bacteria isolated from diabetic foot ulcers were biofilm producers and presented resistance to commonly used antibiotics. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *E. coli* were the species recovered in their investigation. *Staphylococcus aureus* isolates were resistant to ofloxacin, ciprofloxacin, trimethoprim-sulphamethoxazole, and gentamicin but very sensitive to oxacillin and vancomycin. *Pseudomonas aeruginosa* isolates showed resistance to the commonly used antibiotics such as ofloxacin, cefotaxime, ampicillin, ceftazidime and imipenem. *E. coli* and *Klebsiella pneumoniae* isolates were fully sensitive to amikacin. In their study, 8 strains of *S. aureus* were moderate biofilm producers and three *S. aureus* strains (1, 2 and 7) were non-biofilm producers. Out of 11 *Pseudomonas* species isolates studied for biofilm formation, 2 isolates were strong biofilm producers, 7 isolates were moderate biofilm producers, and 2 isolates were weak biofilm producers. All *Enterobacteriaceae* strains were moderate biofilm producers with exception for *K. pneumoniae* which were strong biofilm producer.²⁶

Thokur S Murali et al. did another study. In their investigation, they examined for microbial associations in diabetes and non-diabetic foot ulcers/wounds and evaluated the bacteria for antibiotic sensitivity and ability to form biofilms. *Staphylococci*, *Pseudomonas*, *Citrobacter* and *Enterococci* are major colonizers in diabetic samples. Though non-diabetic samples had a similar assemblage, the frequency of occurrence of distinct groups of bacteria varied. Gram-negative bacteria were shown to be more frequent in diabetic wound ulcers, whereas Gram-positive bacteria were found to be more prevalent in non-diabetic ulcers. In their investigation, 355 microorganism strains from diabetes and non-diabetic people were examined for their propensity to produce biofilms. 159 of the 355 isolates had the ability to form high to moderate levels of biofilms. The remaining 65 strains were classed as moderate biofilm producers, while 94 strains were classified as high biofilm producers. The other strains were classified as non/weak biofilm producers.²⁷

The majority of the biofilm-forming bacteria in their investigation were multidrug-resistant (resistance to more than three antibiotics). *Pseudomonas aeruginosa* was resistant to all antibiotics tested, and two strains were found to produce modest amounts of biofilm while one strain produced significant levels of biofilm. These three *Pseudomonas aeruginosa* strains were all isolated from diabetic wounds. Though all the 23 strains of *Acinetobacter*

were found to be multidrug-resistant, only nine strains were found to be high biofilm producers. In the case of bacteria isolated from non-diabetic samples, more than 50% of Staphylococci were high biofilm producers and were also multidrug-resistant. Only in the case of *E. coli*, there was a significant difference in the biofilm production observed in isolates between diabetic and non-diabetic wounds, even though the numbers of strains tested were lower in the case of non-diabetic wounds compared with diabetic wounds.²⁷

Kiana Karimi et al. conducted another investigation to assess the antibiotic resistance pattern and biofilm development in *Klebsiellapneumoniae* strains. Samples from urinary tract, wound, blood, tracheal tube aspirate, throat and sputum samples, and abscess drain cultures were included in their study. According to their findings, 92% of the isolates had high-level resistance to cefotaxime, while all of the isolates were susceptible to colistin. Biofilm formation was observed in most of the isolates. There was a substantial link between biofilm growth and antibiotic resistance. In their investigation, they grouped *Klebsiella pneumoniae* isolates that produced biofilm strains into four types. According to the biofilm study, 27 (32.5%) *K. pneumoniae* isolates produced weak biofilms, 18 (21.6%) isolates produced moderate biofilms, and 17 (20.4%) isolates produced robust biofilms. Furthermore, blood culture samples developed biofilm weakly more than others, and endotracheal tube aspirates exhibited the highest moderate biofilm formation.²⁸

Another study by Sarah Mohammed Mohsin on molecular investigation of virulence genes of *Klebsiella pneumoniae* Isolated from diabetic foot infections in Karbala Governorate to determine the virulence of biofilm-forming *Klebsiella pneumoniae* that infect diabetic foot infections in Karbala city by isolating these species and identifying them molecularly showed that out of 119 isolates from 142 diabetic foot ulcer patients, 25 were identified as *Klebsiella pneumoniae*. All isolated *K. pneumoniae* were 100% resistant to gentamycin and levofloxacin, with 96% resistance to ciprofloxacin and 92% resistance to amikacin. According to the microtiter plate technique, 60% of *K. pneumoniae* isolated from diabetic foot infections in their investigation were high producers of biofilm. PCR revealed that 88% of *K. pneumoniae* isolates had the *mrkD* gene, 100% carried the *Cps* gene, 12% carried the *K1* gene, and 60% carried the *K2A* gene. *Klebsiella pneumoniae* from diabetic foot ulcers vary genetically, provide resistance to therapeutically important antibiotics, and carry virulence characteristics.²⁹

Another study conducted by Mohamed Salah et al. discovered that 62% of bacterial isolates from diabetic wounds were *Staphylococcus* species followed by *Pseudomonas* species, *E. coli*, and *Klebsiella* species. In their study, *Staphylococcus* species showed the highest susceptibility to oxacillin, followed by vancomycin, and all species showed resistance to cefotaxime. Some *Klebsiella* sp. showed susceptibility to vancomycin, cefotaxime, and ceftioxin, with resistance to oxacillin and methicillin. Amazingly, all isolated *Pseudomonas* sp. showed resistance to all antibiotics except one isolate that showed susceptibility to cefotaxime. *Bacillus* species isolated were resistant to cefotaxime and ceftioxin. Furthermore, *E. coli*

isolated showed susceptibility to all antibiotics tested. Almost all organisms isolated were biofilm producers.³⁰

B.V.S Lakshmi et al. conducted another investigation to determine the spectrum of multidrug-resistant bacteria linked with diabetic foot ulcers, their antibiotic sensitivity pattern, and to detect biofilm formation. A total of 100 samples were gathered from individuals with persistent diabetic foot ulcers for their investigation. 80 isolates were obtained from the samples. There were 28 Gram positive organisms and 52 Gram negative organisms isolated. The most often isolated organisms were *Pseudomonas aeruginosa* and *Staphylococcus aureus* followed by *E. coli*, *Klebsiella pneumoniae* and Methicillin Sensitive *Staphylococcus aureus*, *Enterococcus*, *Proteus mirabilis*, *Citrobacter*, and *Acetobacter*. 93.75% of the isolates were multidrug resistant and most of them exhibiting biofilm formation.³¹

In a study by Jin Xin Zheng, 250 *K. pneumoniae* bacteremia isolates were collected from patients in Shenzhen and Shanghai, China. The presence of virulence genes in their genomes was identified using PCR. The isolates were subjected to multilocus sequence typing (MLST) and clonal complex (CC) categorization based on housekeeping genes. Crystal violet staining was used to detect biofilms. In their study, found that virulence genes such as *magA* (*K1*) or *wcaG*-positive isolates exhibited a greater biofilm formation ability than *hmv* or *K2A* (*K2*)-positive isolates. Biofilm formation was more pronounced in isolates that were positive for virulence factors, such as *magA*(*K1*), *aero*, *rmpA*, and *rmpA2*, than in isolates that were negative for those virulence factors.³²

Another research by Asli Vatan et al. found that the total rate of biofilm production among 339 wound isolates from diabetic foot infection was 34% and that the biofilm production rate was substantially greater in gram-negative microorganisms than Gram positives. The maximum biofilm generation was shown by *Acinetobacter baumannii* (62%), followed by *P. aeruginosa* (52%), and *Klebsiella* spp. (40%).³³

Ramaprabha et al. conducted another study to determine the bacteriome and mycobiome of diabetic ulcers, and associated biofilm formation and anti-microbial resistance profile of the pathogens, as well as the molecular characterization of biofilm-forming resistant isolates using PCR. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most often isolated pathogens among aerobic bacteria, among anaerobes, and *Candida albicans* among fungi. Gram negative bacteria showed high sensitivity to piperacillin/tazobactam, meropenem, and gram positive cocci to vancomycin and linezolid. 82% of bacterial isolates and 50% of fungal isolates were biofilm producers. *Staphylococcus aureus* was a strong biofilm producer. On molecular characterisation; *mecA* genes were present in resistant biofilm-forming isolates.³⁴

Saba Noor et al. found that, out of 50 specimens obtained from infected diabetic foot ulcers, 74% of cases were affirmative by bacteriological assays and 90% were positive by polymerase chain reaction (PCR). Among the processed

samples, 44 isolates were detected by phenotypic analysis and 65 microorganisms by species-specific PCR. *Klebsiella pneumoniae* was the most common pathogen found, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.³⁵

Tiria et al. investigated 102 clinical *Klebsiella pneumoniae* isolates for virulence genes and performed phenotypic study of serum resistance, biofilm formation, and hypermucoviscosity. The virulence genes *mrkD*, *ybtS*, *wcaJ*, *entD*, and *rmpA* exhibited prevalence values of 95.1%, 30.4%, 27.5%, 22.5%, and 0.98%, respectively. More than half of the isolates were strong biofilm producers. The *mrkD* and *ybtS* genes were shown to be substantially associated with the biofilm-forming phenotype. 11.8% of the isolates exhibited the hypermucoviscous phenotype, suggesting hypervirulence and they were positive for the *mrkD* gene.³⁶

In another study by Hongdong Lil et.al, denaturing gradient gel electrophoresis (DGGE) was used to characterize the bacterial species in the biofilms on the Endotracheal tubes (ETT) and species-specific PCR was used to detect common oropharyngeal *Streptococcus* species and known ETT associated pathogens. DGGE profiling of ETT biofilms showed multiple banding patterns indicating a diverse bacterial community. They used molecular methods such as the 16S rRNA PCR-DGGE method to characterize polymicrobial communities. Bacterial species detected in their study included *Klebsiella* sp., *Streptococcus* sp., *Pseudomonas* sp., and *Bacillus* sp., Oropharyngeal bacteria were found in 25 of 29 samples. In 10 of 20 neonates, the polymicrobial communities on the ETT specimens included at least one *Streptococcus* species, *P. aeruginosa* and *K. pneumoniae*.³⁷

Mohammed Zubair conducted another research. 97 gram-negative bacilli were isolated from 57 individuals, with mixed bacterial illness (67.8%) and monomicrobial infection (32.2%). *Escherichia coli* was the most common (42.2%) isolate followed by *Pseudomonas aeruginosa* (23.7%), *Klebsiella oxytoca* (11.3%), *Klebsiella pneumoniae* (9.2%), *Proteus vulgaris* (5.1%), *Acinetobacter* species (5.1%), *Proteus mirabilis* (2%) and *Morganella morganii* (1.0%). According to their findings, biofilm-producing organisms infected 77.1% of diabetic foot ulcer patients.³⁸ A study conducted in India revealed that *E. coli* was the predominant pathogen observed in infected diabetic lesions, followed by *Staphylococcus aureus*.³⁹

4. Discussion

Foot ulcer is a major problem in diabetes which if left untreated, results in limb amputation. Diabetic individuals tend to develop several complications such as neuropathy and peripheral arterial disease that contribute to foot ulceration.

The current study focuses on the molecular basis of biofilm formation and antibiotic resistance of bacteria that cause diabetic foot ulcers, particularly *Klebsiella* species. According to the findings of this review, gram-negative organisms are the major colonizers in diabetic ulcer along with gram-positive organisms and the intensity of biofilm

development in antibiotic-resistant bacteria was greater than in sensitive strains, and a remarkable correlation between antibiotic resistance and biofilm formation was discovered. Several studies have shown that chronic wounds harbor biofilm-forming microbes.⁴⁰

Dr.S Chavan et al. observed in their study that out of the 94 gram-negative organisms isolated from diabetic ulcers, 20.9% were *Klebsiella* species, with the majority (53.6%) being ESBL-producing strains. In another investigation, *Klebsiella pneumoniae* isolated from diabetic illnesses was resistant to carbapenems in two cases, with twofold resistance to fluoroquinolones but not to co-trimoxazoles, according to Giovanni Boschetti et al. In another study by Jean-Marie Liesse Iyamba et.al, *Klebsiella pneumoniae* from diabetic ulcer were fully sensitive to amikacin and they were strong biofilm producers.

A study by Kiana Karimi et.al, concluded that a correlation between antibiotic resistance and biofilm formation. *Klebsiella pneumoniae* strains isolated were categorized based on their biofilm formation, weak biofilm producers, moderate biofilm producers and strong biofilm producers. All strains were highly resistant to cefotaxime and highly susceptible to colistin. Another result of a study by Sarah, was that *Klebsiella pneumoniae* isolated from diabetic foot infection were highly resistant 100% towards gentamycin and levofloxacin, followed by 96% resistance toward ciprofloxacin and 92% resistance toward amikacin. While studying the virulence of biofilm-forming *Klebsiella*, they found out that 88% of *K. pneumoniae* isolates contained the *mrkD* gene, 100% contained the *Cps* gene, 12% contained the *K1* gene, and 60% contained the *K2A* gene. Moreover in other study, predominant organism causing diabetic ulcer were *Staphylococcus aureus* followed by *Pseudomonas* species, *E. coli* and *Klebsiella* sp. 60% of these isolated *Klebsiella* were biofilm positive and they were resistant to oxacillin and methicillin.

Shimaa A Abdel Salam et.al. concluded that hypervirulent *K. pneumoniae* were strong biofilm producers and showed distribution of *rmpA* genes. This result in agreement with Guo et al. and Lin et al. stated that *rmpA* gene was detected in 85.7%, 80.7% of strains of *HvKp* respectively.^{41, 42}

40% of *Klebsiella* sp. were isolated from diabetic foot infection and were biofilm producers according to Asli Vatan et.al. In contrast, in another study by Ramaprabha et.al *Klebsiella* species were not isolated from diabetic foot ulcers. The study conducted in India revealed that *E. coli* and *Klebsiella* were the most frequently identified isolates.⁴³ In microbiology, it is well observed that diabetic wounds that have been infected tend to exhibit a polymicrobial nature.⁴⁴

According to Asmaa M Elbrolosy et.al. and Tiria et.al., *mrkD* gene were identified as a genetic determinant of biofilm formation by *K. pneumoniae* and *HvKp*. From the results of the study by Amir and Reza the *fimH*, *mrkA* and *mrkD* were detected in *K. pneumoniae* isolated from all types of samples and most of the isolates were biofilm producers.

HvKp strains had considerably lower rates of resistance to popular antibiotics compared to *cKp* strains. Additionally,

ESBL synthesis was more prevalent in cKp strains than hvKp strains. The hvKp isolates were substantially related with moderate and strong biofilm formation, and they had considerably greater capsular serotype-specific genes K1 and K2 and showed that *rmpA*, *rmpA2* and *magA* were significantly associated with hvKp strains in study by Mohammed Talal Alharbi et.al. A prior study found a substantial connection between these genes and hvKp, but not with cKp.

5. Conclusion

In conclusion, there is a remarkable correlation was observed between antibiotic resistance and biofilm formation. The strength of biofilm development varied between samples. Understanding the molecular pathways involved in biofilm homeostasis is critical for preventing biofilm development and eradicating existing ones. Antibiotic resistance is a major public health concern, particularly in underdeveloped countries. It is critical to emphasize that the study's capacity to show the changing patterns of bacterial resistance over time may be restricted. This is because the susceptibility of bacteria to antibiotics may fluctuate due to the appearance of new strains and alterations in the use of antibiotics.

Consent

It is not applicable.

Ethical Approval

It is not applicable.

Competing Interests

The authors have declared that no competing interests exist.

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