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BIOFILM FORMATION IN *ENTEROBACTER SPP.* ISOLATED FROM DIFFERENT CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL IN BANGLADESH

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ABSTRACT

Background: *Enterobacter spp.* are important emerging causes of nosocomial and community-acquired infections. Biofilm formation is an important virulence factor of *Enterobacter spp.* Formation of biofilm by *Enterobacter spp.* often results in multidrug-resistant chronic infections. **Aim:**This study aimed to phenotypically determine biofilm formation in *Enterobacter spp.* isolated from various specimens in the laboratory, and to correlate this biofilm formation with the patterns of antimicrobial resistance observed in the isolated *Enterobacter*. **Materials and Method:** From September 2018 to July 2019, this study was conducted in the Microbiology department of Bangladesh Medical University (BMU) in Dhaka, Bangladesh. A total of 50 laboratory isolates of *Enterobacter* from various clinical specimens were collected and evaluated for biofilm production using the Tissue Culture Plate (TCP) method. Additionally, the isolates were tested for antimicrobial susceptibility using the Kirby-Bauer disc diffusion method. The study also assessed the antimicrobial resistance patterns of biofilm-producing and non-producing isolates. **Results:** Among the 50 *Enterobacter* isolates, 32 (64%) were found as biofilm producers phenotypically.The rates of antimicrobial resistance were higher in biofilm-producing isolates of *Enterobacter* compared to those that do not produce biofilms. Resistance rates were relatively higher among the group of antibiotics known as cephalosporins. Resistance rates to cefoxitin, cefixime, cefuroxime, and ceftazidime were 81%, 66%, 63%, and 50%, respectively, in biofilm-producing isolates. In contrast, the resistance rates were 72%, 50%, 33%, and 33%, respectively, in non-biofilm-producing isolates. A relatively low level of resistance was observed against gentamicin, amikacin, netilmicin, meropenem, and piperacillin-tazobactam. **Conclusion:** The production of biofilm is a significant virulence factor of *Enterobacter spp.*, playing a crucial role in pathogenesis and resistance to antimicrobial agents. Detection of biofilm by the tissue culture plate method can be established as a routine laboratory test. This would guide the physicians to prescribe appropriate antibiotics and to take other necessary measures to combat biofilm-related infections.

Keywords: Antimicrobial resistance, Bangladesh, Biofilm, Enterobacter, Virulence factor.

INTRODUCTION

Enterobacter species are Gram-negative bacteria that belong to the family Enterobacteriaceae.. The bacterium comprises part of the normal flora of the gastrointestinal tract of 40%–80% of the human population and is widely distributed in the environment[1].*Enterobacter spp*can cause opportunistic infections, including respiratory tract infection, urinary tract infection, wound infection, skin and soft tissue infection, ophthalmic infections, and bloodstream infections. They are especially known for causing infections related to catheters, as they have been observed forming biofilms on indwelling catheters in hospitalized patients[2,3].

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A biofilm is a group of microbial cells that securely adheres to a surface and is encased in a matrix predominantly made of polysaccharides[4].Biofilms enhance the survival of bacterial populations in hospital settings and inside patients, which increases the likelihood of hospital-acquired infections[5,6]. According tothe National Institutes of Health (NIH), biofilms are accountable for over 65% of hospital-acquired infections and 80% of all other microbe-related infections.

The present study aimed to phenotypically assess biofilm production among 50 *Enterobacter spp*. isolates and to determine its association with antimicrobial resistance. Understanding the pathogenecity of *Enterobacter spp*is crucial, as bacteria in biofilms show greater resistance against antimicrobial agents. Several factors contribute to this resistance, including reduced absorption of antibiotics through biofilm matrix, transferof resistance through genes amongst populations of bacteria in biofilms, metabolic inactivity of bacteria within biofilms, and inactivation of drugs by multiple mechanisms, etc[7] .

Consequently, effective treatment of biofilm-related infections is often highly challenging. Therefore, this study aimed to phenotypically analyse the production of biofilm by 50 *Enterobacter* isolates by utilizing the TCP method.The relationship between the production of biofilm and antimicrobial resistance was also evaluated.

MATERIALS AND METHOD

This cross-sectional study was conducted at the Department of Microbiology and Immunology, BMU, from September 2018 to July 2019.

Collection and identification of organisms:

Enterobacter isolates were collected from various clinical specimens, including urine, pus, wound swabs, sputum, blood, and body fluids, at the Laboratory of Microbiology Department, BMU. The Enterobacter were identified based on their colony morphology and biochemical tests.

Detection of biofilm production using the TCP Method:

TCP is a quantitative test for biofilm detection,which was conducted following the methods outlined by Toledo[8].ATCC strain 700603 *Klebsiella pneumoniae* was used as a positive control, and fresh Brain Heart Infusion Broth (BHIB) was utilized as a negative control for biofilm formation.

Day 1: Three to four well-isolated identical colonies of *Enterobacter* from MacConkey agar were inoculated in a test tube containing 10 mL of BHIB containing 0.25% glucose and were incubated aerobically at 37^0 C for 18 hours without shaking.

Day 2: The culture in BHIB was then diluted by adding fresh BHIB containing 0.25% glucose. The ratio was 1 to 40. Thesterile flat-bottom polystyrene microtiter plate (Greiner Bio-One International, Kremsmunster, Austria) contains 96 wells, and each of the wells was filled with 200 µl of the diluted broth culture of each test organism in triplicate. As a positive control, the ATCC strain 700603, *Klebsiella pneumoniae,*was incubated, diluted, and then added to the microtiter plate in the same manner.As a negative control, sterile BHIB was added[9]. The microtiter plate was then kept in the incubator at 37°C for 24 hours.

Day 3: The contents of each well in the microtiter plate were flicked off by gently tapping on a blotting paper in an inverted position. Each well was washed three times with 200 µl of Phosphate buffer solution (PBS) with a one-minute interval during each washing step. Following every washing step, the wells were emptied by gently tapping on an absorbent paper to remove any residual buffer. The microtiter plate was dried in an inverted position, and 200 µl of 2% formalin was added to each well for fixation of adherent material. The plate was stored in the refrigerator at 4º C

for one hour. Then the solution was flicked off and blotted. Then, staining of the wells was done with 200 µl per well of 1% Crystal violet (CV) for 15 minutes. Following this, and wells were washed with deionized water in order to remove any excess stain. Then the Crystal Violet was solubilized by adding 200µl ethanol-acetone (in an 80:20 v/v ratio) to each well.

Calculation of optical density (OD) value:

The interpretation of the formation of biofilm was done following the criteria of Stepanovic[10].
Each test was performed three times, and the average optical OD values were calculated for all tested strains, the positive control, and the negative control.

The cut-off value (ODc) was of three standard deviations (SD) above the mean OD of the negative control: ODc = average OD of negative control + (3×SD of negative control).

The final OD value of a tested strain is expressed as (OD = average OD of a strain - ODc). ODc value is calculated for each microtiter plate. If the OD value was negative, it was presented as zero.Any positive OD value was considered indicative of biofilm production.

TCP is a quantitative and dependable method to identify biofilm-forming organisms. When compared to the Tube Method (TM) and Congo Red Agar (CRA) methods, the TCP methodis recommended as a general screening technique for identifying biofilm-producing bacteria in laboratories.

Antimicrobial susceptibility test:

The disk diffusion method was applied for antimicrobial susceptibility testing of the isolated organisms, employing the "Kirby-Bauer method"[11]. Mueller-Hinton agar and commercially available antibiotic discs from Oxoid Ltd (UK) were utilized. The disc contents and the zones of inhibition were analyzed according to the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2019)[12].

RESULTS

A total of 50 *Enterobacter*strains were obtained from various clinical specimens. Among these, 34 (68%) *Enterobacter* were obtained from urine, 7 (14%) from blood, 4 (8%) from wound swab, and 1(2%) isolate each from pus, sputum, tracheal aspirate, Cerebrospinal Fluid, and peritoneal fluid. The majority of *Enterobacter* were isolated from urine (Table 1).

Out of 50 *Enterobacter* isolates, 32(64%) were found as biofilm producers and 18 (36%) were non-biofilm producers by the tissue culture plate method (Figure 1)

Enterobacter that form biofilms were isolated from various clinical specimens..The majority of the *Enterobacter* (n=34) were obtained from urine, out of which 22(64.7%) were biofilm producers. Out of 7 blood isolates 5(71.4%) were biofilm producers (Table 2)

Table 3 shows the antimicrobial resistance pattern of biofilm-producing isolates and non-biofilm-producing isolates of *Enterobacter spp*. The rates of resistance were generally higher in biofilm producers for nearly all tested antibiotics.

Table-1: Distribution of *Enterobacter spp.* isolates among different clinical specimens (n=50)

Type of sample	No. of isolates (%)
Urine	34 (68%)
Blood	7 (14%)
Wound swab	4 (8%)
Pus	1 (2%)
Sputum	1 (2%)
Tracheal aspirate	1 (2%)
CSF	1 (2%)
Peritoneal fluid	1 (2%)
Total	50

n=Number of *Enterobacter* isolates

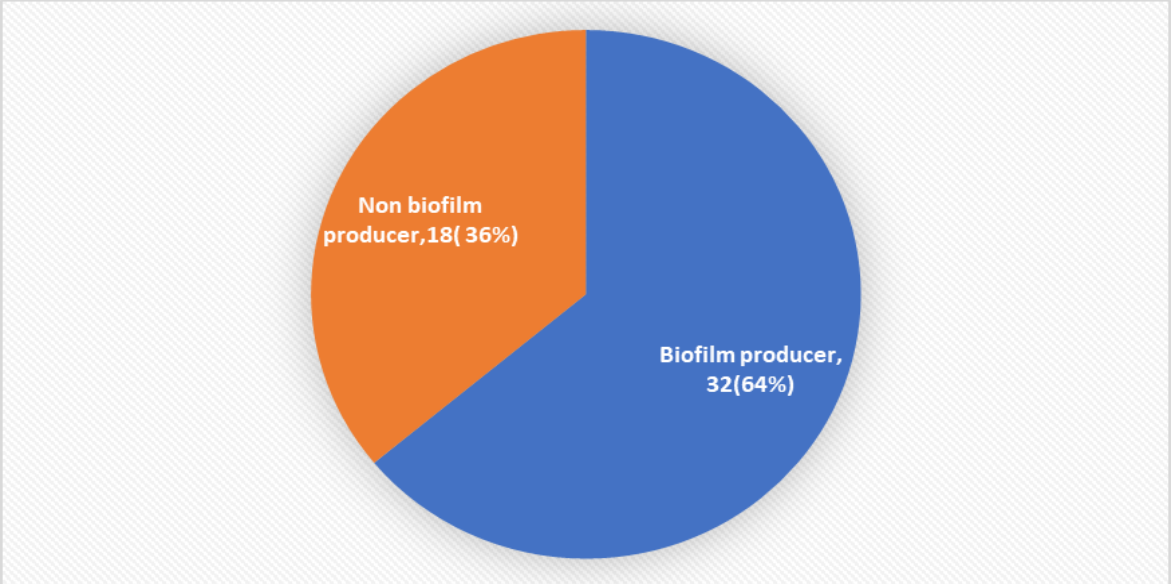

Figure 1: Shows the rate of biofilm production in *Enterobacter spp.* isolates (n=50)

Table 2: Detection of biofilm formation of *Enterobacter spp* in different clinical specimens (n=50)

Type of specimen	Biofilm producing isolates n(%)
Urine (n=34)	22 (64.7)
Blood (n=7)	5 (71.4)
Wound swab (n=4)	3 (75)
Pus (n=1)	1 (100)
CSF (n=1)	00
Sputum (n=1)	00
Body fluid (n=1)	00
Tracheal aspirate (n=1)	1 (100)

n=Number of *Enterobacter* isolates

Table 3: Antimicrobial resistance pattern of biofilm-producing and non-biofilm producing *Enterobacterspp.* Isolates (n=50)

Antimicrobial agent	Biofilm-producing resistant isolates n (%) n=32	Non-biofilm producing isolates n (%) n=18	Total n (%) n=50
Ceftazidime	16 (50)	6 (33)	22 (44)
Cefuroxime	20 (63)	6 (33)	26 (52)
Cefoxitin	26 (81)	13(72)	39 (78)
Cefixime	21 (66)	9 (50)	30 (60)
Ceftriaxone	16 (50)	6 (33)	22 (44)
Co-trimoxazole	11 (34)	6 (33)	17 (34)
Ciprofloxacin	12 (38)	5 (28)	17 (34)
Nalidixic acid	17 (53)	6 (33)	23 (46)
Nitrofurantoin (Urinary isolates only)	18 (56)	9 (50)	27 (54)
Gentamicin	9 (28)	5 (28)	14 (28)
Amikacin	9 (28)	10 (56)	19 (38)
Aztreonam	13 (41)	6 (33)	19 (38)
Meropenem	7 (22)	7 (39)	14 (28)
Netilmicin	9 (28)	4 (22)	13 (26)
Piperacillin-tazobactam	11 (34)	3 (17)	14 (28)
Ticarcillin-clavulanate	21 (66)	7 (39)	28 (56)

n=Number of *Enterobacter* isolates

DISCUSSION

Enterobacter are found as normal flora of the gastrointestinal tract, and they are also found as commensals in the surrounding environment. In recent years, *Enterobacter* has become a significant cause of infections acquired in hospital settings.

The majority of the *Enterobacter* isolates, 34(68%), were obtained from urine, followed by 14% from bloodin this study. This finding is similar to the finding reported in Iran, where 51.6% *Enterobacter* were obtained from urine and 11.9% *Enterobacter* were obtained from blood[13]. In this study, a high rate of biofilm production (64%) was observed among the *Enterobacter* isolates.

Among the biofilm-positive isolates, 22 (68.8%) were isolated from urine samples, followed by 15.6% from blood, 9.4% from wound swabs, and 3.1% from pus and tracheal aspirate, i.e., urinary isolates have the highest percentage of biofilm production. This may be because biofilms form on catheters, causing catheter-associated urinary tract infections. Bacteria also adhere to uroepithelium, forming biofilm[14].Hassan et al.reported 94 % of urinary *Enterobacter* isolates as biofilm producers[15]. Sabir et al. reported in another study that 87.5% of the urinary *Enterobacter* were biofilm producers[16].

This study examined the relationship between biofilm formation and antimicrobial resistance. It was found that biofilm-producing isolates exhibited higher rates of antimicrobial resistance compared to those that did not produce biofilm. Notably, higher resistance rates were observed in the cephalosporin group of antibiotics. The resistance rates recorded for cefoxitin, cefixime, cefuroxime, and ceftazidime in biofilm-producing isolates were 81%, 66%, 63%, and 50%, respectively. Whereas, in non-biofilm producing isolates, the rates of resistance were 72%, 50%, 33% and 33% respectively. Relatively lower resistance rates were observedin the Aminoglycoside group, as well as in meropenem and piperacillin-tazobactam,for both biofilm-producing and non-producing bacteria. Nahar et al also noted higher resistance to antimicrobial in biofilm producing bacteria in comparison to the non-biofilm producing isolates[17] .It is well evident that biofilm raises drug resistance by slow penetration, altered growth rate, reduced metabolism, and altered gene expression. Some organisms also express biofilm-specific resistance genes not required for biofilm formation but promote antibiotic resistance[18,19].

CONCLUSION

Enterobacter is emerging as an important health concern owing to its increasing rate of infection,especially among hospitalized patients. Biofilm production is an important virulence factor of *Enterobacter spp*., contributing significantly to its pathogenesis and remarkable antimicrobial resistance. Therefore, newer therapeutic approaches and antibiotics with antibiofilm activity are essential to confront these pathogens. The Tissue culture plate method is recommended for use as a screening technique for detecting biofilm in the laboratories. This would be helpful to prescribe an appropriate combination of antibiotics to treat biofilm-related infections.

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CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Paterson DL, Rossi F, Baquero F, Hsueh PR, Woods GL, Satishchandran V, et al. In vitro susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections worldwide: the 2003 Study for Monitoring Antimicrobial Resistance Trends (SMART). J Antimicrob Chemother. 2005;55(6):965-73. doi: 10.1093/jac/dki117.

2. Storti A, Pizzolitto AC, Pizzolitto EL. Detection of mixed microbial biofilms on central venous catheters removed from intensive care unit patients.Braz JMicrobiol. 2005; 36(3): 275–280.doi /10.1590/S1517-83822005000300013

3. Mokracka J, Koczura R, Pawłowski K, Kaznowski A. Resistance patterns and integron cassette arrays of Enterobacter cloacae complex strains of human origin. J Med Microbiol. 2011;60(Pt 6):737-743. doi: 10.1099/jmm.0.027979-0. 21330416.

4. DonlanRM. Biofilms: Microbial Life on Surfaces. Emerg Infect Dis. 2002; 8: 881-890. http://dx.doi.org/10.3201/eid0809.020063

5. Lebeaux D, Ghigo J, Beloin C. Biofilm-Related Infections: Bridging the Gap between Clinical Management and Fundamental Aspects of Recalcitrance toward Antibiotics. Microbiol Mol BiolRev.2014;78(3): 510-43. https://doi.org/10.1128/mmbr.00013-14

6. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Medical Microbiol. 2015;64(Pt 4):323–34. doi: 10.1099/jmm.0.000032

7. LiuHY, Prentice EL, Webber MA. Mechanisms of antimicrobial resistance in biofilms. npj Antimicrob Resist. 2024; 2(1). doi:10.1038/s44259-024-00046-3.

8. Toledo-Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M, et al. The enterococcal surface protein, Esp, is involved in Enterococcus faecalis biofilm formation. Appl Environ Microbiol. 2001; 67(10):4538-45. doi: 10.1128/AEM.67.10.4538-4545.2001.

9. Singh AK, Yadav S, Chauhan BS, Nandy S, Singh R, Neogi K, et al. Classification of Klebsiella pneumoniae based on their in vitro Biofilm Forming capabilities and Elucidation of the Biofilm Matrix Chemistry With Special Reference to the Protein content. Front Microbiol. 2019; 10:669. doi:103389/fmicb.2019.00669

10. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007;115(8):891-9. doi: 10.1111/j.1600-0463.2007.

11. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol.1966 ; 45(4):493-96.

12. CLSI (2017) Performance standards for antimicrobial susceptibility testing. 27th ed,CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne.

13. Afshar ZM, Miladi R, Janbakhsh A, Mansouri F, Sayad B,Vaziri S, et al. The Prevalence and Pattern of Enterobacter Antibiotic Resistance in the Patients Admitted to Imam Reza Hospital in Kermanshah, Iran (2016-2018). J Kermanshah Univ Med Sci. 2021; 25(1): e112518. doi: 10.5812/jkums.112518.

14. Soto SM. Importance of biofilms in urinary tract infections: new therapeutic approaches. Adv Biol. 2014; 5:1-13. https://doi.org/10.1155/2014/543974 .

15. Hassan R, El-Naggar W, El Sawy E, El Mahdy A . Characterization of some virulence factors associated with *Enterbacteriaceae* isolated from urinary tract infections in Mansoura Hospitals. Egyptian Journal of Medical Microbiology. 2011; 20(2):9-18.Available from:https://www.researchgate.net/publication/324983247 [(Accessed on 8th August 2025)].

16. Sabir N, Ikram A, Zaman G, Satti L, Gardezi A, Ahmed A, et al. Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance. American journal of infection control. 2017; 45(10):1101-05. Am J Infect Control. 2017 ;45(10):1101-1105. doi: 10.1016/j.ajic.2017.05.009.

17. Nahar A, Anwar S,MiahMRA. Association of Biofilm Formation with Antimicrobial Resistance Among the Acinetobacter Species in A Tertiary Care Hospital in Bangladesh. J Med.2013; 14(1), 28–32. https://doi.org/10.3329/jom.v14i1.14533.

18. Patel R. Biofilms and Antimicrobial Resistance. Clin Orthop Relat Res. 2005 ;(437):41-7. doi: 10.1097/01.blo.0000175714.68624.74.

19. Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial Biofilm and its Role in the Pathogenesis of Disease. Antibiotics (Basel). 2020 ;9(2):59.doi: 10.3390/antibiotics9020059.