BIOFILM PRODUCTION BY UROPATHOGENS LIKE KLEBSIELLA SPP AND PSEUDOMONAS SPP AND THEIR ANTIBIOTIC SUSCEPTIBILITY

Mahato S[1], Mandal P[2], Mahato A[3]*

Affiliation

1. *AASRA Research and Education Academy Counsel, Biratnagar-6, Nepal*
2. *Department of Microbiology, Mahendra Morang Adarsh Multiple Campus, Tribhuvan University, Nepal*
3. *Lecturer, Department of Orthopaedics, Birat Medical College and Teaching Hospital, Biratnagar, Nepal*

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**** Corresponding Author***

Mr. Sanjay Mahato

AASRA Research and Education Academy Counsel

Biratnagar-6, Nepal

Email: mahato.sanjay@gmail.com

ORCID ID: https://orcid.org/0000-0002-0154-8129

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ABSTRACT

Introduction

Urinary Tract Infection (UTI) causes inflammation which is a common, painful and sometimes life-threatening condition as well. Despite high prevalence of bacteriuria, the information on biofilm forming bacteria is negligible.

Objectives:

This study aims at understanding the status of the biofilm forming nature of *Klebsiella* spp and *Pseudomonas* spp and their drug resistance property with several class of antibiotics with a prime focus on resistance pattern against few penicillin based drugs to few empirical drugs in today's time.

Methodology:

Urine samples were analyzed and the isolates were biochemically identified. Then, the isolates were tested for several drugs so as to identify multidrug resistance nature of isolates by Kirby-Bauer Disc method. Biofilm forming nature was examined on Congo Red Agar.

Results

Out of 35 urine samples, 13 isolates (37.1%) were found to be positive with significant bacteriuria. Eight samples (22.8%) showed incidence of *Klebsiella* spp and 5 samples (14.3%) showed *P. aeruginosa*. The prevalence of *Klebsiella* spp. (*Klebsiella pneumoniae* (46.2%) and *K. oxytoca* (15.4%)) and *P. aeruginosa* was found to be 61.4% and 38.6% respectively. 66.6% of *K. pneumoniae* and 50% *K. oxytoca* were biofilm forming pathogen. *K. pneumoniae* and *K. oxytoca* were resistant to amoxycillin, amoxycillin-clavulanate, and cefoxitin; while were sensitive to nitrofurantoin and azithromycin. *P. aeruginosa* were sensitive to azithromycin (100%), but showed 60% resistance to levofloxacin and ofloxacin. Eight (61.5%) isolates were found to be MDR. 100% of *Klebsiella oxytoca* (n=2), 66.7% of *Klebsiella pneumoniae* (n=4), and 40% of *Pseudomonas aeruginosa* (n=2) were multidrug resistant (MDR). Multiple antibiotic resistance (MAR) indices of bacteria revealed that all the 13 isolates were Multi-Antibiotics Resistance strains.

Conclusion

Biofilm forming nature is now much greater in *Klebsiella* spp; while most of the isolates like *Klebsiella* and *Pseudomonas* are multidrug resistant.

KEYWORDS

Antibiotic, biofilm, klebsiella, pseudomonas, susceptibility, uropathogen.

INTRODUCTION

Urinary Tract Infection (UTI) with a pathogen causes inflammation which is a common, distressing and occasionally life-threatening condition. UTI affects people of all ages and both genders. Female are more susceptible to UTIs compared to male.[1] Worldwide about 150 million people are diagnosed with UTIs each year.[2,3] In sexually active healthy female patients with structurally and functionally normal urinary tracts may have uncomplicated UTIs.[4] Complicated UTIs are associated with co-morbid conditions that prolong the need for treatment like abnormalities of the urinary tract, foreign body presence like indwelling catheter or stone, and infection with MDR pathogens.[5] Signs and symptoms may include fever, chill, dysuria, urinary urgency, frequent and cloudy or malodorous urine.

Infection are almost always ascending in origin and caused by the bacteria inhabiting the distal gastrointestinal tract and colonizing the perineal area. Urinary tract infections are caused by a variety of gram-negative and gram-positive bacteria. The gram-negative bacteria include a large number of aerobic bacilli such as *Escherichia* spp*, Klebsiella* spp*, Pseudomonas* spp*, Enterobacter* spp*, Citrobacter* spp*, Salmonella* spp*, Proteus* sppetc. and the gram-positive bacteria includes *Staphylococcus* spp*, Streptococcus* spp and *Enterococcus* spp.[6] About 80% of acute uncomplicated UTIs are caused by *E. coli*, 10-20% by *Staphylococcus saprophyticus* and 5% or less are caused by other Enterobacteriaceae such as *Klebsiella* spp*, Proteus* spp, or *by Enterococcus* spp.[7,8] The most common causes of complicated UTIs are *Klebsiella* spp, *Pseudomonas* spp and *Proteus* spp.[8]

Biofilm are complex communities of surface associated cells enclosed in a polymer matrix containing water channels.[9] A biofilm can be composed of a single species or a big conglomerate of microbial species which protects microorganisms from opsonization, antibodies, phagocytosis and removal via the ciliary action of epithelial cell.[10,12] The emergence of antibiotic resistance in the management of urinary tract infections is a serious public health issue.

In Biratnagar, there are reports of high prevalence of bacteriuria, but information on biofilm forming bacteria is negligible. Based on this dearth, the present study was designed to determine the spectrum of uropathogens in cases of UTI in the Koshi Zonal Hospital (which serves majority of the population of the eastern districts of Nepal) and to determine the antimicrobial susceptibility pattern and biofilm forming nature of the isolates. Our study also aimed at the drug resistance property of pathogens like *Klebsiella* and *Pseudomonas* with a prime focus on resistance against penicillin based drugs to few empirical drugs of today's time.

METHODOLOGY

Study Area and Sample Collection

Clean catch midstream urine samples were collected from both inpatients and outpatients of the Koshi Zonal Hospital, Biratnagar (Nepal). Diversified population (urban and rural) visit the hospital because of low cost treatment by this government hospital. The duration of the study was four months (February-May 2017) and was carried out in the Microbiology Laboratory of the Department of Microbiology at Mahendra Morang Adarsh Multiple Campus (MMAMC), Biratnagar, Nepal. Thirty-five clinical samples of urine were processed during the study. Research and ethical approval were taken from AASRA Research and Education Academy Counsel, Biratnagar. Informed consent was obtained from all participants.

About 15 to 20 ml urine specimen was collected in a 20 mL sterile wide mouthed, screw-capped universal container. The container comprising specimen was appropriately labeled with unique sample number, date, and time of collection. After collection, it was transported to the Microbiology laboratory of MMAMC, Biratnagar for culture, drug susceptibility testing and biofilm formation testing. The specimen was analyzed within two hours of collection.

Sample Cultureand Identification of Organisms

Processing of samples were done by a previously described methodology. Patients that presented positive urine culture ($\geq 10^5$ CFU/mL) were studied. Significant bacteriuria was defined as colony count $\geq 10^5$ CFU/mL. Each sample was aseptically inoculated (in triplicate) into MacConkey agar plates (Himedia, Mumbai, India) and cetrimide agar plates (Himedia, Mumbai, India). The plates were incubated aerobically at 37^{o}C for 18-24hr. The colonial characteristics of bacterial isolates were observed and sub-cultured.

The resulting isolates were subjected to microscopic examinations like Gram staining, capsule staining and appropriate biochemical tests for proper identification. Gram negative isolates were further identified by different biochemical tests like oxidase, catalase, motility, indole and H_2S production, MR-VP, citrate utilization, urea hydrolysis, triple sugar iron utilization tests. The identity of bacteria was, thus, established based as described in the book of Cheesbrough.[13]

Biofilm production (Congo Red Agar method)

Congo Red Agar (CRA) method was used for biofilm detection. For this, Congo Red Agar media was prepared with brain heart infusion agar (HiMedia, Mumbai, India) and Congo Red indicator. Congo Red stain was prepared as a concentrated aqueous solution and autoclaved at 121^{o}C for 15 minutes separately from other medium constituents. It was, then, added to the autoclaved brain heart infusion agar at 55^{o}C and transferred to the petri-plates. CRA plates were, then, inoculated with fresh isolated pure culture and incubated at 37^{o}C for 24 hours. After incubation, CRA plates were observed whether an organism forms a black colony or not.[13]Well identified *K. pneumoniae* and *Pseudomonas* strains were used as standard. The standard strains were biofilm non-forming and biofilm forming and were used as negative and positive control respectively to compare the result.

Antibiotic Susceptibility Study

Antibiotic susceptibility testing of the bacterial isolates was

achieved by disc diffusion method (Kirby-Bauer method) on Muller-Hinton agar (Himedia, Mumbai, India) and interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines.[14] A homogenous suspension of 0.5 MacFarland standard of a pure colony was prepared in 5 mL of sterile normal saline (0.85% NaCl). The bacterial suspension was evenly distributed over the entire surface of Mueller-Hinton Agar (MHA) plates using a sterile swab. The antibiotic disc (Himedia, India) containing the following antibiotics was used: Amoxycillin (AMX, 10 µg), Amoxycillin/ Clavulanate (AMC) (20/10 µg), Cefotaxime (CTX, 30 µg), Cefoxitin (CFX, 30 µg), Ofloxacin (OF, 5 µg), Levofloxacin (LE, 5 µg), Azithromycin (AZM, 15 µg), and Nitrofurantoin (NIT, 300 µg). Once the discs were applied onto MHA plates, the plates were incubated at 37^{0}C for 24 hr. Zone of inhibition was measured and interpreted using the standard chart and the organisms were reported as susceptible, intermediate, or resistant accordingly.[14, 15] Since *Pseudomonas* is intrinsically resistant to Amoxycillin, Cefoxitin, and Nitrofurantoin, those antibiotics were not used. *Pseudomonas* were tested with only five remaining drugs while *Klebsiella* were tested with eight drugs.[14] Well identified sensitive strains of *Klebsiella* and *Pseudomonas* were used as control.

The criterion for Multidrug Resistance

All those isolates which demonstrated the resistance to at least one agent in three or more classes of the drug were defined as multidrug resistant (MDR).[15]

Multiple antibiotic resistance (MAR) index

MAR index is a number of antibiotics to which test isolate displayed resistance divided by the total number of antibiotics to which the test organism has been evaluated for sensitivity. So, MAR index for each isolate was calculated as per the recommendation of Krumperman.[16] Data frequencies and cross tabulations were used to summarize descriptive statistics.

RESULT

Isolation and Identification

Out of 35 urine samples, 13 isolates (37.1%) were found to be positive with significant bacteriuria, and no sample showed polymicrobial bacteriuria. Of 35 urine samples, 8 samples (22.8%) showed incidence of *Klebsiella* spp. and 5 samples (14.3%) showed *P. aeruginosa*. No growth was seen in rest of the samples. The prevalence of *Klebsiella* spp. and *P. aeruginosa* was found to be 61.4% and 38.6% respectively.

Eight isolates were from MacConkey Agar plate and 5 were taken from Cetrimide Agar plate. All isolates were identified using cultural, morphological and biochemical characteristics. Upon morphological examination, all the isolatesfrom MacConkey Agar plate when sub-cultured on Nutrient Agar (NA) which upon microscopic examination were found to be capsulated gram-negativebacilli. On the contrary, isolates from Cetrimide Agar plate were non-capsulated gram-negative bacilli. Biochemical test confirmed that 6 isolates ($U_{2,}$ $U_{4,}$ $U_{5,}$ $U_{10,}$ $U_{25,}$ U_{26}) of MacConkey plate were *Klebsiella pneumoniae*, 2 isolates ($U_{14,}$ U_{18}) were *Klebsiella oxytoca*,and 5 isolates (U_{3}, $U_{19,}$ $U_{20,}$ $U_{21,}$ U_{31}) were *Pseudomonas aeruginosa.*

Biofilm formation

Out of 13 isolates, only 5 isolates (4 isolates ($U_{2,}$ $U_{5,}$ $U_{25,}$ U_{26}) of *K. pneumoniae* and 1 isolate(U_{18}) of *K. oxytoca*) formed black colonies on Congo Red Agar plate which showed biofilm forming positive isolates; while all of the *Pseudomonas aeruginosa* were biofilm non-forming.66.6% of *K. pneumoniae* and 50% of *K. oxytoca*showed biofilm positive.

Figure 1: Biofilm formation by *K. pneumoniae*

Antibiotic Susceptibility Test

K. pneumoniae and *K. oxytoca* were resistant to amoxycillin, amoxycillin-clavulanate, and cefoxitin; while were sensitive to nitrofurantoin and azithromycin. All *K. pneumoniae* showed resistance to cefoxitin and amoxycillin; while 83.3% were resistant to Cefotaxime and 66.7% were resistant to levofloxacin and ofloxacin. All of *K. oxytoca* showed resistance to cefoxitin, amoxycillin, cefotaxime, levofloxacin, and ofloxacin. *P. aeruginosa* were sensitive (100%) to azithromycin. All of *P. aeruginosa* were resistant to amoxycillin-clavulanate. 60% of *P. aeruginosa* showed resistance to levofloxacin and ofloxacin.

Table 1. Multiple antibiotic resistance (MAR) indices of *Klebsiella* and *Pseudomonas*

Resistant antibiotics	Number of Drug used	Sample	Strains	MARI	Biofilm
AMC + AMX + CFX	8	U_2	*Klebsiella pneumoniae*	0.37	Forming
AMC + LE + OF	5	U_3	*Pseudomonas aeruginosa*	0.6	Non-forming
AMC+AMX+CFX+CTX+LE+OF	8	U_4	*Klebsiella pneumoniae*	0.75	Non-forming
AMC + AMX + CFX + CTX	8	U_5	*Klebsiella pneumoniae*	0.5	Forming
AMC+AMX+CFX+CTX+ LE+OF	8	U_{10}	*Klebsiella pneumoniae*	0.75	Non-forming
AMC+AMX+CFX+CTX+ LE+ OF	8	U_{14}	*Klebsiella oxytoca*	0.75	Non-forming
AMC+AMX+CFX+CTX+LE+OF	8	U_{18}	*Klebsiella oxytoca*	0.75	Forming
AMC + CTX	5	U_{19}	*Pseudomonas aeruginosa*	0.4	Non-forming
AMC + CTX + LE + OF	5	U_{20}	*Pseudomonas aeruginosa*	0.8	Non-forming
AMC + CTX	5	U_{21}	*Pseudomonas aeruginosa*	0.4	Non-forming
AMC+AMX+CFX+CTX+ LE+OF	8	U_{25}	*Klebsiella pneumoniae*	0.75	Forming
AMC+AMX+CFX+CTX+ LE+OF	8	U_{26}	*Klebsiella pneumoniae*	0.75	Forming
AMC + CTX + LE + OF	5	U_{31}	*Pseudomonas aeruginosa*	0.8	Non-forming

Out of 13 isolates, 8 (61.5%) were found to be MDR. 100% of *Klebsiella oxytoca* (n=2)($U_{14,}$ U_{18}), 66.7% of *Klebsiella pneumoniae* (n=4)($U_{4,}$ $U_{10,}$ $U_{25,}$ U_{26}), and 40% of *Pseudomonas aeruginosa* (n=2) ($U_{20,}$ U_{31}) were multidrug resistant (MDR) as these strains were resistant to at least one antibiotic of three or more classes (Table 1).

Multiple antibiotic resistance (MAR) indices of bacteria revealed that all the 13 isolates had a MARI of greater than 0.2 giving 100% incidence of Multi-Antibiotics Resistance strains. Of all the *Klebsiella*, 12.5% (n=1) had MARI as 0.37 and 0.5 each, 80% (n=6) had MARI value of 0.56. Of all *Pseudomonas*, 1(20%) was resistant to 3 drugs (MARI = 0.6) while 2 strains (40%) were resistant to 2 drugs(MARI=0.4) and rest of 40% were resistant to 4 drugs (MARI=0.8) (Table 1).

DISCUSSION

In this study *Klebsiella* spp. (61.4%) and *P. aeruginosa* (38.6%) show higher prevalence compared to the work of Fatima et al and Djordjevic et al who reported 24% of *Klebsiella* and 10.5% of *P. aeruginosa* respectively.[17,18] This is probably because *Klebsiella* forming capsule are able to produce a potent urease which acts on urea to produce ammonia, rendering the urine alkaline.[19]*Klebsiella* species has a number of virulence factors, including fimbrae, capsule, iron scavening systems, and urease.[20]

Biofilm production and antibiotic resistance is of a great concern in the treatment of disease and infections. Biofilm highly promotes recurrent and persistent infections, which leads to high mortality rates, prolonged treatments and causes high cost in health care services. The result of biofilm production of the uropathogens showed *K. pneumoniae* has high ability for biofilm production. 66.6% of *K. pneumoniae* and 50% of *K. oxytoca* showed biofilm positive. Biofilm producing bacteria showed much greater resistance to antibiotics than their free-living counterparts.[21] This study showed 83.3% of *Klebsiella* species were biofilm positive while all the isolates of *P. aeruginosa* were biofilm negative which deviated with the findings of Maharjan et al and Yang who found 20% *P. aeruginosa* were biofilm positive and 44.7% *Klebsiella*spp respectively.[22,23]

This study revealed a higher prevalence rate of resistant to the commonly prescribed antibiotics. *Klebsiella* were highly resistant to amoxycillin, amoxycillin/clavulanate, cefotaxime and cefoxitin. This could be due to the production of ESBLs capable of conferring bacterial resistant to the penicillin, first, second and third generation cephalosporin such as cefotaxime and cefoxitin. They exhibit resistance to antibiotics by various method like restricted penetration of antibiotics into biofilm, decrease growth rate and expression of resistance genes.[24] The study revealed *Pseudomonas aeruginosa* were not tested with amoxycillin, cefoxitin, and nitrofurantoin because of their intrinsic resistance towards these drugs.[25] Efflux pumps are common component of multidrug resistant *P. aeruginosa* and they prevent accumulation of antibiotic within the bacterium, extruding the drugs from the cell before they have the opportunity to achieve an efficient concentration at the site of action.[26] *Klebsiella pneumoniae* showed 100% resistance to amoxycillin which agreed (100%) with the findings of Beyene and Tsegaye.[27] Resistant to antimicrobial agents include: intrinsic resistance, altered permeability barriers across bacterial outer membranes, preventing uptake of the compounds by inhibiting its corresponding transport carriers, modifing the target's binding sites so that it no longer recognizes the antibiotics, forming biofilm, mutation and also the ability to enzymatically degrade the antibiotics.[26,28] Around 61.5% of isolates were found to be MDR. 75% of *Klebsiella* spp and 40% of *Pseudomonas aeruginosa*were MDR which was much lower than Singh et al.[29] MARI of all isolates were greater than 0.2 giving 100% incidence of Multi-Antibiotics Resistance strains which was in total agreement with Oli et al.[30]

CONCLUSION

While most of the isolates like *Klebsiella* and *Pseudomonas* are multidrug resistant, biofilm forming nature is now much greater in *Klebsiella* spp.

RECOMMENDATIONS

A regular supervision and broad scale cross sectional study of MDR and biofilm forming have to be done so as to have a clear idea regarding the changes in the nature of bacteria which will guide the medical practitioner about the use of empirical treatment.

LIMITATIONS OF THE STUDY

Due to unavailability of standard strains for quality control, the ATCC standard strains were not used. More sample size might have supported data strongly.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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REFERENCES

1. McLellan LK, Hunstad DA. Urinary Tract Infection: Pathogenesis and Outlook. Trends Mol Med. 2016;22(11):946–957.
2. Karishetti MS, Shaik HB. Clinicomicrobial assessment of urinary tract infections in a tertiary care hospital. Indian J Health Sci Biomed Res 2019;12:69-74
3. Panday DR, Amar A, Subedi A, Hussain Md S, Gupta M, Rauniar GP. Antibiotic Usage and its Culture Sensitivity Pattern in Urinary Tract Infections at Tertiary Hospital in Eastern Nepal. Kathmandu Univ Med J. 2017;60(4):332-5.
4. Mody L, Juthani-Mehta M. Urinary tract infections in older women: a clinical review. JAMA. 2014;311(8):844–854.
5. Cortes-Penfield NW, Trautner BW, Jump RLP. Urinary Tract Infection and Asymptomatic Bacteriuria in Older Adults. Infect Dis Clin North Am. 2017;31(4):673–688.
6. Kline KA, Lewis AL. Gram-Positive Uropathogens, Polymicrobial Urinary Tract Infection, and the Emerging Microbiota of the Urinary Tract. Microbiol Spectr. 2016;4(2):10
7. Ehlers S, Merrill SA. Staphylococcus Saprophyticus. [Updated 2019 Mar 27]. In: Stat Pearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482367/
8. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269–284.
9. Jhajharia K, Parolia A, Shetty KV, Mehta LK. Biofilm in endodontics: A review. J Int Soc Prev Community Dent. 2015;5(1):1–12.
10. Tilahun A, Haddis S, Teshale A, Hadush T. Review on Biofilm and Microbial Adhesion. International Journal of Microbiological Research. 2016;7(3):63-73.
11. Seifi K, Kazemian H, Heidari H, et al. Evaluation of Biofilm Formation Among Klebsiella pneumoniae Isolates and Molecular Characterization by ERIC-PCR. Jundishapur J Microbiol. 2016;9(1):e30682.
12. Cheesbrough M. Biochemical tests to identify bacteria. In: District Laboratory Practice in Tropical Countries, Part II. 2nd ed. New York: Cambridge University Press; 2009. p. 45–58.
13. Bellifa S, Hassaine H, Terki IK, Didi W, M'hamedi I, Lachachi M, et al. Study of Biofilm Production and Antimicrobial Resistance Pattern of Klebsiella Pneumoniae Isolated from Urinary Catheter at the University Hospital of Tlemcen. American Journal of Microbiology and Biotechnology. 2016;3(2):13-17.
14. Clinical and Laboratory Standards Institute. 2016. Performance standards for antimicrobial susceptibility testing: 26th informational supplement. Document M100-S26. CLSI, Wayne, USA.
15. Mahato S, Mahato A, Yadav J. Prevalence and Identification of Uropathogens in Eastern Nepal and Understanding Their Antibiogram Due to Multidrug Resistance and ESBL. Asian Pacific Journal of Microbiology Research, 2018;2(1):09-17.
16. Krumpermann PH. Multiple Antibiotics Resistance Indexing of E. coli to Identify High Risks Sources of Faecal Contamination of Foods. Applied and Environmental Microbiology. 1983;46(1):165-170.
17. Fatima S, Muhammad IN, Usman S, Jamil S, Khan MN, Khan SI. Incidence of multidrug resistance and extended-spectrum beta-lactamase expression in community-acquired urinary tract infection among different age groups of patients. Indian J Pharmacol. 2018;50(2):69–74.
18. Djordjevic Z, Folic MM, Zivic Z, Markovic V, Jankovic SM. Nosocomial urinary tract infections caused by Pseudomonas aeruginosa and Acinetobacter species: sensitivity to antibiotics and risk factors. Am J Infect Control. 2013;41:1182–7.
19. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269–284.
20. Paczosa MK, Mecsas J. Klebsiella pneumoniae: Going on the Offense with a Strong Defense. Microbiol Mol Biol Rev. 2016;80(3):629–661.
21. Vuotto C, Longo F, Balice MP, Donelli G, Varaldo PE. Antibiotic Resistance Related to Biofilm Formation in Klebsiella pneumoniae. Pathogens. 2014;3(3):743–758.
22. Yang D, Zhang Z. Biofilm-forming Klebsiella pneumoniae strains have greater likelihood of producing extended-spectrum beta-lactamases. J. Hosp. Infect. 2008;68:369–371.
23. Maharjan G, Khadka P, Shilpakar GS, Chapagain G, Dhungana GR. "Catheter-Associated Urinary Tract Infection and Obstinate Biofilm Producers," Canadian Journal of Infectious Diseases and Medical Microbiology, vol. 2018, Article ID 7624857, 7 pages.
24. Rewatkar AR, Wadher BJ. "Staphylococcusaureus and Pseudomonasaeruginosa-Biofilm formation Methods". Journal of Pharmacy and Biological Sciences. 2013;8(5):36-40.
25. Pang Z, Raudonis R, Glick BR, Lin TJ, Cheng Z. Antibiotic resistance in Pseudomonasaeruginosa: mechanisms and alternative therapeutic strategies. Biotechnol Adv. 2019; 37(1):177-192.
26. Dreier J, Ruggerone P. Interaction of antibacterial compounds with RND efflux pumps in Pseudomonas aeruginosa. Front Microbiol. 2015;6:660.
27. Beyene G, Tsegaye W. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in jimma university specialized hospital, Southwest Ethiopia. Ethiop J Health Sci. 2011;21(2):141-6.
28. Eyo AAO, Ibeneme EO, Thumamo BDP, Asuquo AE. Antibiotic resistance profiles of clinical and environmental isolates of Pseudomonas aeruginosa in Calabar, Nigeria. IOSR Journal of Pharmacy and Biological Sciences. 10(4):I,09-15.
29. Singh VK, Tuladhar R, Chaudhary MK. Beta Lactamase Producing Escherichiacoli, Klebsiellapneumoniae and Methicillin Resistant Staphylococcusaureus among Uropathogens. Nepal Journal of Science and Technology. 2015;16(1):105-112.
30. Oli AN, Eze DE, Gugu TH, Ezeobi I, Maduagwu UN, Ihekwereme CP. Multi- antibiotic resistant extended-spectrum beta-lactamase producing bacteria pose a challenge to the effective treatment of wound and skin infections. Pan Afr Med J. 2017;27:66.