Original article

Prevalence of drug-resistant microbes in sepsis cases of catheter and fistula based haemodialysis

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ARTICLE INFO

ABSTRACT

Background: Chronic stage renal disease is a severe disease of the kidney which affects people globally. According to the global burden of diseases in 2010, this disease has caused more deaths worldwide and due to the high death rate, the ESRD (end-stage renal disease) is now ranked up from 27th to 18th range in the list.

Methodology: Dialysis samples were collected from the Haripur city and surrounding areas. Samples were inoculated on different selective media for bacterial growth. In addition, different biochemical tests were also performed for identification, where as the resistance genes were identified through a polymerase chain reaction.

Result: Out of the total 100 dialysis patient's blood samples, only 17 showed the presence of gram-positive bacteria i.e., *Staphylococcus aureus* while two shown the presence of gram-negative bacteria i.e., *Klebsiella pneumoniaeee* and *Pseudomonas aeruginosa*. While in molecular identification two antibiotic resistance genes *muc* and *mec*A belong to the *staphylococcus* strain shown their presence.

Conclusion: A high infection rate has been observed in fistula-based hemodialysis (17(77.27%)) as compares to catheter-based hemodialysis (5(22.3%) with no significant difference of incidence between the groups (p > 0.05).

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1. Introduction

There are different causative agents of infection in hemodialysis patients. The most common of which were pathogens i.e., *Staphy-*

lococcus aureus. The Staphylococcus infections were most common in hemodialysis patients because of its complication causing tissue, wound infections, meningococcal septicemia, and in severe cases, it is hard to treat infections. The rate of death and illness was also increased because of the *Staphylococcus aureus*. The *Staphylococcus aureus* infection in dialysis was found to be 1.45–66.0% of all the infections in dialysis (Mylotte and Tayara 2000). The bacteremia in dialysis patients was mainly due to the *Staphylococcus aureus* i.e., 27–39% (Danese et al., 2006). The infections in hemodialysis also occurred due to the gram-negative bacteria. The infection by gram-negative bacteria increases by 25% in hospital and hemodialysis blood infection (Albrecht et al., 2006). The rate of infection from different gram-negative bacterial strains was different, for example, Klebsiella 11.1%, Pseudomonas 5.1%, *E. coli* 49%, Enter-

obacter 13.1%, and Proteus mirabilis 6.1%. Due to transfusion of blood in dialysis, there were chances of developing viremia in patients including HCV and HBV viral infection. Bacteremia and viremia were the co-morbidities in dialysis patients. In the hemodialysis and peritoneal dialysis, there was another virus BKV that is also common and infect almost 90% of the patient population. In immunocompromised patients, the chances of reactivation of BKV virus are higher (Sharif et al., 2015). Therefore, the purpose of the current study was to find the prevalence of drug-resistant bacterial strains in hemodialysis patients.

End-stage renal disease (ESRD)is one ofthe major diseases that inflict a heavy economic burden on the globe (Weiner, 2009). In chronic kidney disease (CKD), the ratio of ESRD has been increased much during the last few decades and the only option of treatment has been limited to dialysis or a kidney transplant. The CKDhas high prevalence rates among adults (Gorodetskaya et al., 2005; Kimmel and Patel, 2006) but it also affects children (Fadrowski et al., 2006). The CKD in the pre ESRDtreatment phase demands a cost excess of $26,000 per case per year in the USA (Smith et al., 2004). The CKD related mortality has also been reported to be higher compared to other diseases (Snively and Gutierrez, 2004). The incidence and prevalence of CKD and ESRD are increasing at an enormously high rate, consequently showing an increase in blood pressure, aging, and Type II diabetic Mellitus. Disease progression and fatality rate are more in cardiovascular complications and bacteremia in dialysis patients. In hemodialysis, the ratio of mortality has been reported 4% but in the case of peritonitis in peritoneal dialysis, the mortality rate is 16% (Johnson et al., 2009).

2. Methodology

This study was duly approved by ethical and biosafety committee of The University of Haripur before the onset of the study This study confirms the declaration of Helsinki and informed consent were taken from the participants or their legal representatives after the sharing the significance of the study. The hemodialysis patients were selected from District Headquarter Hospital, Haripur, and Kidney Centers of Abbottabad and Rawalpindi Districts. The blood samples were taken from all the dialysis patients of the said areas. There is no restriction of age and gender rather this research study comprised all age groups regardless of gender specificity. This study focused mainly on checking the prevalence rate between male and female patients, insertion devices that were used during dialysis, and the infection rate among them. This study was carried out from June 2018 to February 2019 and during this time interval blood samples were also taken from the patients. A total of 100 patients were selected and blood on dialysis samples was taken from hemodialysis patients by the help of sterile syringes and blood was collected in sterile tubes. After this, these collected samples were transferred to the Microbiology laboratory of the Department of Microbiology, University of Haripur. Selective media were used for culturing the different bacteria strains. The selective media used for Staphylococcus aureus, Klebsiella pneumoniaeee and Klebsiella pneumoniaeee weremannitol agar, eosin methylene blue agar and cetrimide agar, respectively.

3. Biochemical analysis

Biochemical tests were also performed for further confirmations. To check antibiotic resistance Kirby Baur method was used. Antibiotics used to detect antibiotic resistance were amoxicillin-clavulanic acid, cefotaxime oxacillin, ceftriaxone, erythromycin, vancomycin, ceftazidime, tetracycline and aztreonam.

4. Molecular analysis (PCR)

DNA extraction was done by boiling method for chromosomal extraction while in case of plasmid extraction WizPrep plasmid kit method were used. In kit method 1.5 ml of bacterial cell culture in a centrifuge tube and centrifuge 13000 rpm for1 min. Add 200 μl PD1 buffer and vortex it. Then add 200 μl PD2 and 300 μl PD3 buffer stepwise and inverted tube many times. After PD3 buffer centrifuge the tube for 10 min. Take supernatant from microcentrifuge tube to the spin column and centrifuge it. Repeating the spin column and centrifuge step several times. Wash the spin column with 600 μl wash buffer and centrifuge it. Dry the spin column and add 50 μl of elution buffer. After keeping at room temperature for 3 min. Centrifuge the spin column at 13000 rpm for 1 min and elute the DNA.PCR amplification of the resistance genes was done by a reported method in the case of Staphylococcus aureus. The mecA and nuc amplifications were done according to Poulsen et al. (2003) and Zhang et al. (2005). The PCR amplified fragment length for the mecAand nucwas 147 bp and 255 bp, respectively. While in the case of ESBL gene in Klebsiella pneumoniaeeee the primer was user defined. The PCR amplified fragment length for the ESBL related genesTEM1 and blaSHV were 208 and 206 bp, respectively. The primers used for the detection of different antibiotic resistance genes in isolated S aureus are given in Table 1. The primers used for the detection of TEM1 and blaSHV genes in Klebsiella pneumoniaeeee were designed using the online primer designing tool Primer3 (Table 1).

For the PCR reaction mixture in the case of Staphylococcus aureus, a total volume of 25 μl included 16 μl nuclease-free water, 1 μl each of the forward and reverse primer,5μl master mix and 2 μl of the DNA template. While in case of the Klebsiella pneumoniaeeee reaction mixture of 25 μl included 20 μl nuclease-free water, 1 μl each of the forward and reverse primer, 4 μl master mix and 5 μl DNA template. In PCR thermal profile of Staphylococcus aureus and Klebsiella pneumoniaeeee were give below in Table 2.

5. Result

The Staphylococcus aureus on mannitol agar showed yellow colonies and media fermentation further identified the bacteria. Klebsiella pneumonea on cetrimide agar showed pink colonies on eosin blue agar and Pseudomonas aeruginosa showed white colonies on cetrimide agar, but Staphylococcus epidermidis showed white colonies and did not show media fermentation.

Out of 100 hemodialysis patients, 17% patients showed positive Staphylococcus aureus, 2% showed gram-negative bacteria and only 1% case of Staphylococcus epidermidis and 2% Pseudomonas aeruginosa and Klebsiella pneumoniaeee were found during this study. There were 78% of samples that did not show any kind of bacterial growth. The sepsis rate in hemodialysis was found to be 22% out of 100% (Fig. 1).

In these sepsis cases, the catheter and fistula ratio are 5 and 12 in Gram positive bacteria while in the case of Gram-negative Pseudomonas and Klebsiella were 0 and 2 in catheter and fistula while one individual was found to be infected with Staphylococcus epidermidis in fistula based hemodialysis (Table 3).

Out of 100 enrolled patients, there were about 22(22%) patients having dialysis through the catheter and 78% dialysis through fistula based haemodialysis. Sepsis confirm 5(22.7%) in the catheter and case of fistula there were 17(77.27%) (Table 4). In 100 samples of haemodialysis patients, there were 71 males in which 15 (21.1%) were confirmed with sepsis cases and in 29 females there were 7 (24.1%) confirmed cases with sepsis (Table 4). There was no significant difference observed in the distribution of sepsis cases between both gender (p > 0.05) shown in Table 4.

Table 1
PCR primers sequences for the detection of selected resistance genes.

Primer	Gene	Sequence	Product size (bp)	Reference
Staphylococcus aureus				
FP	Nuc	5′-TCAGCAAATGCATCACAAACAG-3′	**255**	Poulsen et al.,2003
RP		5′-CGTAAATGCACTTGCTTCAGG-3′		
FP	Mec	5′-GTG AAG ATA TAC CAA GTG ATT-3′	**147**	Zhang et al.,2005
RP		5′-ATG CGC TAT AGA TTG AAA GGA T-3′		
Klebsiella pneumoniaeee				
FP	TEM 1	5′-GGCGCTTTCTCATAGCTCAC-3′	**208**	
RP		5′-GCCTACATACCTCGCTCTGC-3′		
FP	blaSHV	5′-CTTTCCCATGATGAGCACCT-3′	**206**	
RP		5′-CGCTGTTATCGCTCATGGTA-3′		

Table 2
Thermal profile for a polymerase chain reaction.

Initial Denaturation		95 °C	5 min
Denaturation		95 °C	30 sec
Annealing	Nuc/mecA	55 °C	30 sec
	TEM1 /blaSHV	57 °C	30 sec
Extension		72 °C	30 sec
Final Extension		72 °C	10 min
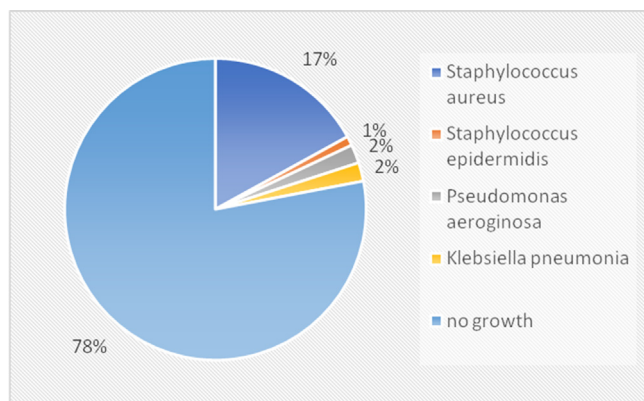
Fig. 1. Graphical representation of bacterial isolates.

In correlations analysis, different risk factors that were associated with each other were also studied. The correlation factors examined were the types of catheter or fistula, family history,

infections during dialysis, and hypertension. Some factors did not show significance value, while some were observed below the average. The family history of kidney disease with HCV co-infection showed significance ($p < 0.01$), infection during dialysis with other infection (sore throat, fever, flu) were also significant ($p < 0.01$). Similarly, hypertension and diabetes were also associated significantly ($p < 0.05$).

The methicillin resistance *Staphylococcus aureus* showed complete resistance to amoxicillin-clavulanic acid, cefotaxime ceftriaxone, erythromycin, aztreonam, and ceftazidime. MRSA showed susceptibility to vancomycin (4) oxacillin (1) and tetracycline (1). The antibiotic resistance patterns are shown in Fig. 2 below graph 2.

The *Pseudomonas aeruginosa* did not show any susceptibility against antibiotics. The antibiotics showing total resistance were amocicalve clavonic acid, cefotaxime, oxacillin, ceftriaxone, vancomycin, aztreonam, tetracycline and ceftazidime. The only erythromycin showed intermediate resistance (Fig. 3).

The *Klebsiella pneumoniaeee* showed resistance to most of the antibiotics that were usedand include amoxiclav clavonic acid, cefotaxime, oxacillin, ceftriaxone, vancomycin, aztreonam, and ceftazidime. But tetracycline showed total susceptibility. And erythromycin showed intermediate susceptibility. The different antibiotic resistance patterns are shown in Fig. 4.

The staphylococcus epidermidis did not show complete resistance against antibiotics. In the case of amoxiclave clavulonic acid, oxacillin, ceftriaxone, aztreonam and ceftazidime shows resistance. But in the case of staphylococcus epidermidis, it did not show susceptibility against any drugs (see Fig. 5).

In the total 17 samples of *S. aureus*, *nuc* was positively detected in 1 sample only, while in the other samples *nuc* was not detected.

Table 3
Catheter and Fistula Based Distribution of Bacterial Species.

Species	All	Fistula	Catheter	χ^2	P
Staphylococcus aureus	17	12(63.1%)	5(22.7%)	0.8	>0.05
Staphylococcus epidermidis	1	1(100%)	0(0%)		
Pseudomonas aeruginosa	2	2(100%)	0(0%)		
Klebsiella pneumoniaeeee	2	2(100%)	0(0%)		

Table 4
Prevalence of Sepsis in Catheter and Fistula Based Haemodialysis and gender.

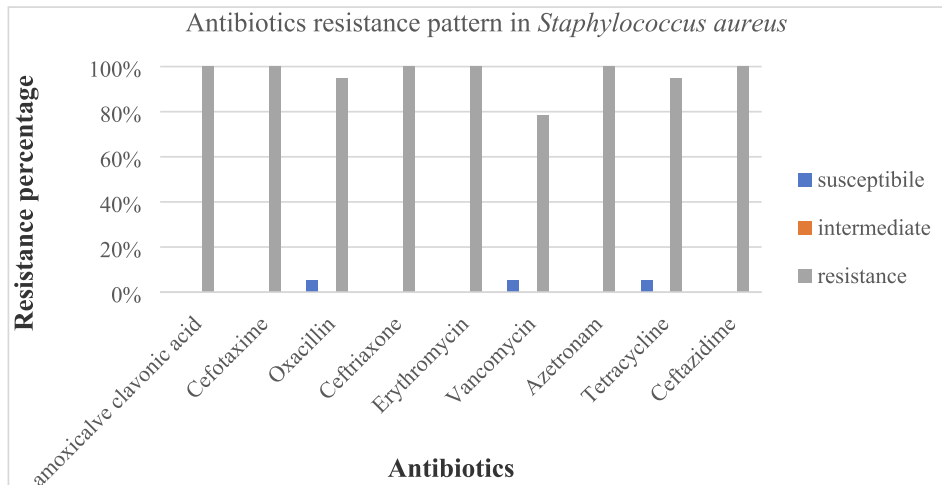|	Sepsis Status		*P-value*	OR(95 %CI)
	Positive N = 22	Negative N = 78		
Mode of route				
Catheter	5(22.7%)	22(28.2%)	>0.05	0.7(0.3–1.9)
Fistula	17(77.27%)	56(71.7%)		
Gender				
Male	15(21.1%)	56(78.8%)	**>0.05**	0.8(0.3–1.9)
Female	7(24.1%)	22(75.8%)		

Fig. 2. Antibiogram of *Staphylococcus aureus* isolates against selected antibiotics.
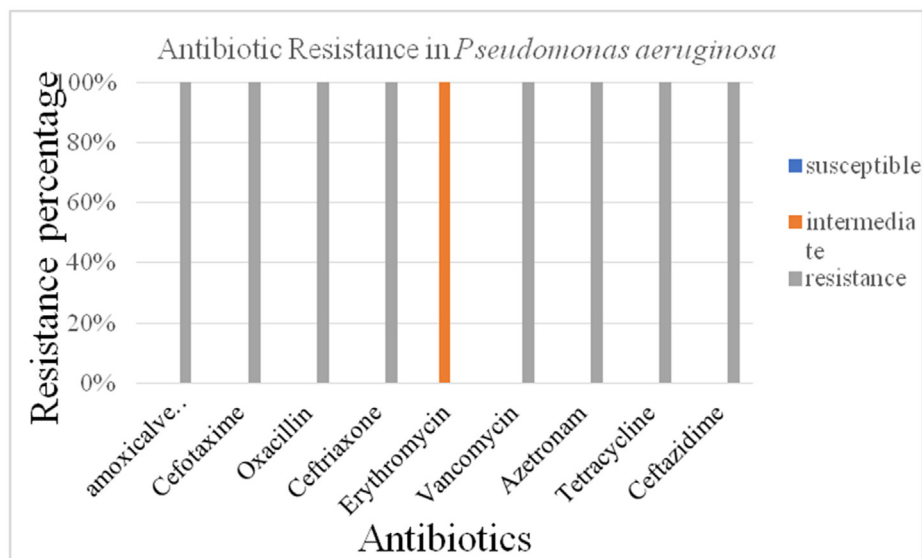

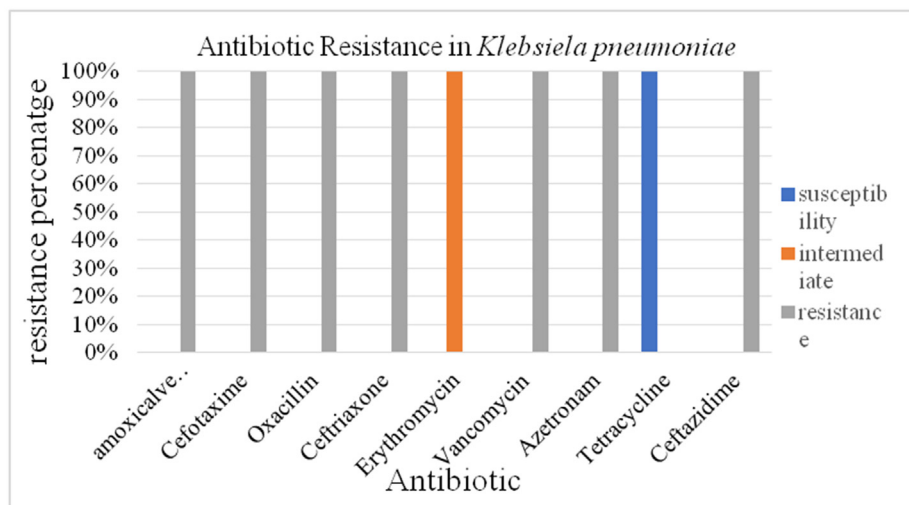**Fig. 3.** Antibiogram in *Pseudomonas aeruginosa* isolates against selected antibiotics.

Fig. 4. Antibiogram in *Klebsiella pneumoniaeee* isolates against selected antibiotics.
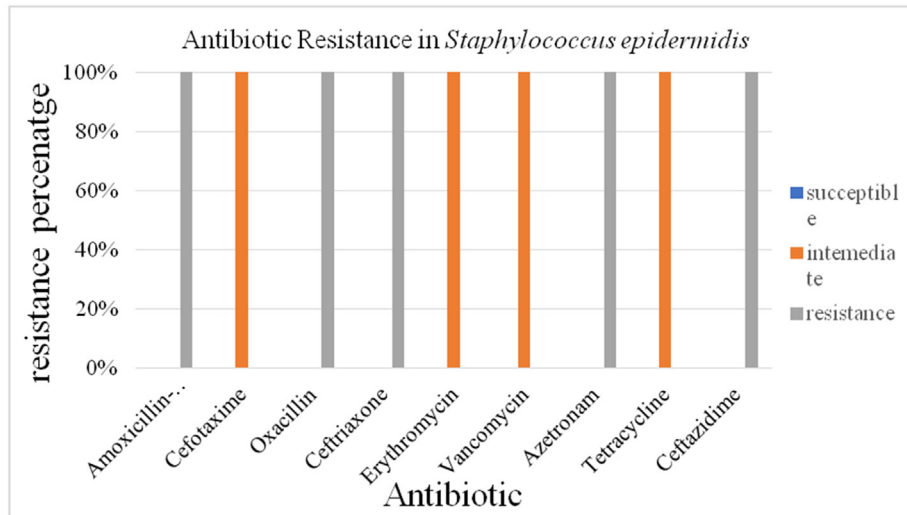
Fig. 5. Resistance pattern of *Staphylococcus epidermidis* isolates against selected antibiotics.

Same is the case of the *mecA*, out of 17 samples only 1 sample the *mecA* was positively detected. The nuc gene detection was not possible in all samples because of the non-optimal experimental condition or the absence of nuc gene in some *Staphylococcus aureus* samples.

The amplification of ESBL related genes from *Klebsiella pneumoniaeeee* has not been observed therefore these strains do not have ESBL gene (see Figs. 6 and 7).
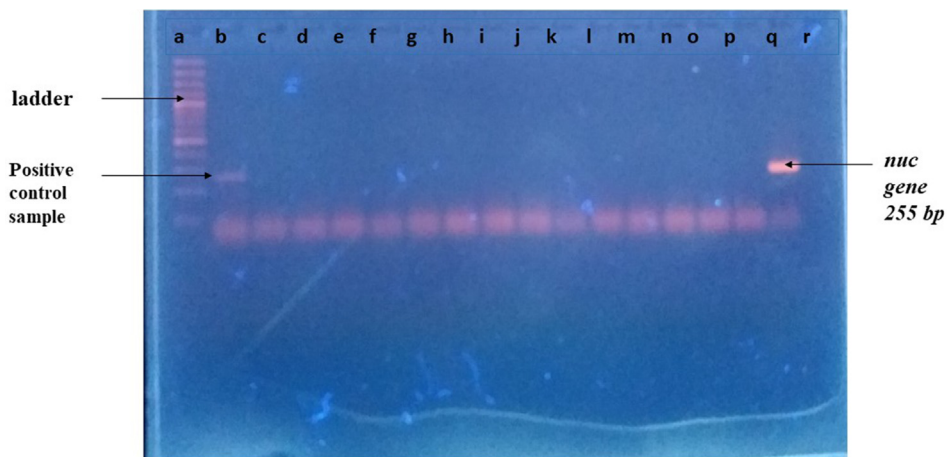

Fig. 6. PCR of nuc gene.

Fig. 7. PCR of mec a gene.

6. Discussion

The CKD, which is currently renowned as a major public health problem, can be detected by simple biochemical tests including creatine base estimate of glomerular filtration rate. Estimates in Asia and Australia indicate that the problem is of the same magnitude in these countries (Chen et al., 2005; Chadban et al., 2003). CKD is considered a dangerous clinical condition for two main reasons: firstly because of the renal impairment which may prelude to the development of ESRD, the disease stage where dialysis and transplantation are needed. Secondly, because it further triggers the risk of developing cardiovascular complications. Independent from these risk factors patients with 4–5 CKD have a death risk for cardiovascular complications which is 2–4 times higher than that of coeval general populations, whilst patients with ESRD have a 100 times higher risk (Baigent et al., 2000).

Sepsis ranks as the tenth leading cause of death in the United States. Sepsis and bacterial infections are very common in ESRD patients, which are followed by cardiovascular diseases, therefore, these infections are the leading cause of death in patients with ESRD (Collins et al., 2014). The diagnosis of sepsis in such patients is often challenging and requires a high index of suspicions. Most studies on sepsis ranging from the sensitivity of systematic response syndrome criteria (Jaimes et al., 2003) to lactate clearance (Nguyen et al., 2004) and optimal fluid therapy have looked in the general population.

Methicillin resistant *Staphylococcus aureus* has become a major public health problem all over the world. It is correlated with incremented morbidity and mortality compared to other pathogenic bacteria. The elevated colonization rates lead to the incrimination of infection rates in the community and medical centers which leads to a significant increase in the treatment costs (Liu et al., 2008). The majority of the researcher in this field suggested that the *mec A* is present in all the MRSA strains and is known to encode penicillin-binding protein 2a (PBP2a) which has a low tropism to all the β lactam antibiotics is the corner stone responsible for producing MRSA phenomenon. Molecular amplification of the *mecA* is recognized as a benchmark to diagnose MRSA in the community as this gene is highly conserved among *Staphylococcus species* (Abbas, 2012).

Recently, a coworker from a tertiary care hospital known to carry MRSA with the *nuc, mecA* duplex PCR method. The result was *nuc* negative MRSA conclusion. Conventional screening of this clinical sample revealed a MRSA positive outcome. This discrepancy could be explained by primer annealing site polymorphism or partial deletion of the nuc gene. These findings are in agreement with our studies, in which only one sample was positive out of 17 samples despite showing antibiotic resistance on the disc diffusion test.

Similarly, *Klebsiella pneumoniaeee* and *Escherichia coli* remain the major ESBL producing organism isolated worldwide, which are recommended to be routinely tested for and reported by the clinical and laboratory standard institute (CLSI 2006). *Klebsiella pneumoniaeee* is one of the most common organisms associated with hospital-acquired infection. On molecular analysis, we could not amplify ESBL related blaSHV and TEM1 genes in *Klebsiella pneumoniaeee* during this study, which indicates that some other related genes may be involved in causing antibiotic resistance in the *Klebsiella pneumoniaeee* strains.

7. Conclusions

In hemodialysis, the chances for survival were low because of a high risk of infections. Physicians control the infection by prescribing antibiotics but their massive use causes the development of antibiotics resistant bacteria. MRSA, the most dangerous bacterial specie and ESBL based gram-negative bacteria cause sepsis in hemodialysis patients. The process of hemodialysis is performed in two ways either by catheter or by fistula, whereas the use of catheter is life-threatening due to venous access. The *mecA* is present in MRSA andthere is no ESBL gene found in *Klebsiella pneumoniaeeee*. Maintenance of hygienic conditions in hemodialysis center and also the precautionary measures must be undertaken by the staff to reduce the chances of infection. The presence of MRSA in haemodialysis patients is an alarming situation to be considered for kidney patients. The CKD is severe and irreversible kidney damage in which kidney is allowed to function by an artificial machine to support life.

8. Recommendations

The infection cases in dialysis centers are on the rise nowadays therefore we suggest the following recommendation.

- Hygienic conditions should be strictly maintained to control the incidence of infections.
- To overcome the infections, the staff must undergo certain mitigating measures like cleaning of the dialysis machine that must be ensured.
- Proper maintenance of the drainage system by the dialysis centers to avoid bacterial spread/contamination.
- To control the antibiotics resistance caused by superbugs, alternative techniques including phage therapy, nanotechnology and small anti-virulence particles can be used to disarm the pathogenic bacteria.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

References

Albrecht, S.J., Fishman, N.O., Kitchen, J., Nachamkin, I., Bilker, W.B., Hoegg, C., Samel, C., Barbagallo, S., Arentzen, J., Lautenbach, E., 2006. Reemergence of gram-negative health care–associated bloodstream infections. Arch. Int. Med. 166 (12), 1289–1294.

Baigent, C., Burbury, K., Wheeler, D., 2000. Premature cardiovascular disease in chronic renal failure. The Lancet 356 (9224), 147–152.

Chadban, S.J., Briganti, E.M., Kerr, P.G., Dunstan, D.W., Welborn, T.A., Zimmet, P.Z., Atkins, R.C., 2003. Prevalence of kidney damage in Australian adults: the AusDiab kidney study. J. Am. Soc. Nephrol. 14 (suppl 2), S131–S138.

Chen, J., Wildman, R.P., Gu, D., Kusek, J.W., Spruill, M., Reynolds, K., Liu, D., Hamm, L. L.E.E., Whelton, P.K., He, J., 2005. Prevalence of decreased kidney function in Chinese adults aged 35 to 74 years. Kidney Int. 68 (6), 2837–2845.

Collins, A.J., Foley, R.N., Chavers, B., Gilbertson, D., Herzog, C., Ishani, A., Johansen, K., Kasiske, B.L., Kutner, N., Liu, J., Peter, W.S., 2014. US Renal Data System 2013 annual data report. Am. J. Kidney Dis. 63 (1), A7.

Danese, M.D., Griffiths, R.I., Dylan, M., Yu, H.-T., Dubois, R., Nissenson, A.R., 2006. Mortality differences among organisms causing septicemia in hemodialysis patients. Hemodialysis Int. 10 (1), 56–62.

Fadrowski, J., Cole, S.R., Hwang, W., Fiorenza, J., Weiss, R.A., Gerson, A., Furth, S.L., 2006. Changes in physical and psychosocial functioning among adolescents with chronic kidney disease. Pediatric Nephrol. 21 (3), 394–399.

Jaimes, F., Garcés, J., Cuervo, J., Ramírez, F., Ramírez, J., Vargas, A., Quintero, C., Ochoa, J., Tandioy, F., Zapata, L., Estrada, J., 2003. The systemic inflammatory response syndrome (SIRS) to identify infected patients in the emergency room. Int. Care Med. 29 (8), 1368–1371.

Johnson, D.W., Dent, H., Hawley, C.M., McDonald, S.P., Rosman, J.B., Brown, F.G., Bannister, K.M., Wiggins, K.J., 2009. Associations of dialysis modality and infectious mortality in incident dialysis patients in Australia and New Zealand. Am. J. Kidney Dis. 53 (2), 290–297.

Kimmel, P.L., Patel, S.S., 2006, January. Quality of life in patients with chronic kidney disease: focus on end-stage renal disease treated with hemodialysis. In: Seminars in Nephrology, vol. 26, no. 1, WB Saunders, pp. 68–79.

Liu, C., Graber, C., Karr, M., Diep, B., Basuino, L.i., Schwartz, B., Enright, M., O'Hanlon, S., Thomas, J., Perdreau-Remington, F., Gordon, S., Gunthorpe, H., Jacobs, R., Jensen, P., Leoung, G., Rumack, J., Chambers, H., 2008. A population-based study of the incidence and molecular epidemiology of methicillin-resistant Staphylococcus aureus disease in San Francisco, 2004–2005. Clin. Infect. Dis. 46 (11), 1637–1646.

Mylotte, J.M., Tayara, A., 2000. Staphylococcus aureus bacteremia: predictors of 30-day mortality in a large cohort. Clin. Infect. Dis. 31 (5), 1170–1174.

Nguyen, H.B., Rivers, E.P., Knoblich, B.P., Jacobsen, G., Muzzin, A., Ressler, J.A., Tomlanovich, M.C., 2004. Early lactate clearance is associated with improved outcome in severe sepsis and septic shock. Crit. Care Med. 32 (8), 1637–1642.

Sharif, A., Sharif, M.R., Aghakhani, A., Banifazl, M., Hamkar, R., Ghavami, N., Eslamifar, A., Ramezani, A., 2015. Prevalence of BK viremia in Iranian hemodialysis and peritoneal dialysis patients. Infect. Dis. 47 (5), 345–348.

Smith, D.H., Gullion, C.M., Nichols, G., Keith, D.S., Brown, J.B., 2004. Cost of medical care for chronic kidney disease and comorbidity among enrollees in a large HMO population. J. Am. Soc. Nephrol. 15 (5), 1300–1306.

Snively, C.S., Gutierrez, C., 2004. Chronic kidney disease: prevention and treatment of common complications. Am. Fam. Phys. 70 (10), 1921–1928.

Weiner, D.E., 2009. Public health consequences of chronic kidney disease. Clin. Pharmacol. Ther. 86 (5), 566–569.