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Molecular investigation of Beta lactamase gene shv in Klebisella pneumonia isolated from diabetic foot patients in Najaf province

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Abstract

The present study included 100 specimens collected of patients suffering for diabetic foot ulcer who attending to Alsader medical city, for both sexes 33-65 y. The results revealed that the frequency among males more than 74(74%) female 26 (26)%. The results revealed that 80 (80) % specimens gave up a positive culture and the other 20 (20)% were negative culture The results showed that G-ve bacteria revealed a high rate 69(69%) , 26(32.5%) K.pneumonia , 19 (23.75%) belonged to E.coli followed by p. aeruginosa 10 (12.5%) then proteus mirabillis 12 (15%) and proteus vulgaris 2(2.5%) , only 8 (10%) isolates belong to S. aureus followed by S.epidermidis 3 (3.75%). K. pneumonia 26(32.5) was one of the bacteria that was isolated, identified, and tested from clinical samples of diabetic foot ulcers in order to phenotypically identify bacteria that were resistant to some antibacterial agents. The results rate of K. peumoniae isolates to Ticarcillin (88.8%), Piperacillin-tazobactam (88.8%) and Colistin (66.6%) were all high. The moderate resistance rate was observed for Trimethoprim- sulfamethoxazole (44.4%). The lowest resistance rate was observed for Cefepime (33.3%), Aztreonam (22.2%), Gentamicin (22.2%), Ceftazidime (11.1%), Meropenem (0%) and Impenem (0%), Levofloxacin (0%), Ciprofloxacin (0%), Amikacin (0%) and Tobramycin (0%). Some virulence factor related with antibacterial resistance were determined such as biofilm production. The results confirmed that 20(76.9%) of K. pneumonia. The present study was demonstrated 15(75%) of isolates were strong to biofilm formation ,4(20%),1(5%) moderate and 0 weak by microtitier plate. On the other hand to study the sensitivity of isolates to several antibiotics were tested via(Kirby-Bauer) disc diffusion way. Using PCR technology and electrophoresis devices, researchers at the molecular level examined the existence of antibiotic-resistant genes (shv gene). PCR analysis revealed that the shv gene was present in 24 (92%) samples.

Keyword

K.pneumoniae, shv gene

%15 of diabetics with foot ulcers do so in an open sore. Typically, they are located at the bottom foot. Diabetes is a metabolic condition that manifests as hyperglycemia and is caused by a problem with insulin action or excretion. in addition to muscular atrophy, neuropathic fractures, and peripheral neuropathy [(1).] The diabetic foot of the bacteriology infection is so convoluted and mostly polymicrobial. It include anaerobes and aerobes. much researchers have offered a image of infection mixing by anaerobic and aerobic bacteria. several bacteria aerobic related to diabetic foot infection contain S. epidedermis, Streptococcus pyogenes, S. saprephyticus, Staphylococcus aureus, Proteus species, Pseudoamonas aerugenosa, Bacillus subtilis, S. mutans, Escherichia coli and Klebsiella pneumoniae. The bacteria anaerobic having Anaerobic Streptococci, Peptostraptococcus species, Clostredium species and Bactariodes fragelis [(2).] infectionof the

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Diabetic foot can be deep or superficial. The deep infection include thebones, superficial fascia, muscles, and joints. This having necrotizing cellulitis cellulitis and wet gangrene. Once the foot ulcer gets to this degree, advised the patient is choice of amputation. The gravity of a diabetic foot infection is classify in to four grades rely on the display [1] K. pneumoniae is one of the utmost significant pathogenic bacteria;it is a Gram negative rod in the Enterobecteriaceae family. The bacterium is find indigenously in soil and waters, but too on mucosal surfaces in mammals, having humans. It is often related at nosocomial infections, that infections acquired through hospitelization [3].

K. pneumoniae, which causes 75% to 86% of infections caused by Klebseiella spp., is the most important pathogen within the genus. Additionally, K. oxytoca, which is responsible for 13% to 25% of infections, is the second-most common Klebseiella species. As human pathogens, Klebsiella spp. may be to blame for nosocomial and community infections [4]. Microorganisms can grow on surfaces and improve biofilms, the compound microbial communities embedded in extracellular matrix or exopolymaric materials counting polysaccherides, nucleic acids and proteins. Biofilms develop survival and growth of microorgenisms due to might reasons [5]. Most recent study suggests that the change in metabolism of bacteria within a biofilm compared to planktonic bacteria has a main part in the antibiotic resistance of biofilms [6]. E.sBLs are beta-lactamases albolite of conferring bacterial resistance to the Penecillins, early and extended-spectrum cephelosporins, and aztraonam [but no cephemycins , carbepenems] via hydrolysis of those antibiotics, inhibited via betalactamase inhibitors like clavulanic acid, sulbectam , tazobectam. This improvement was possible due to the selective pressure exerted via lactam-products soil organisms find in the environment. The utmost common ESBLs are shv-, TEM-, and CTX-M [7].

Specimens' collection and bacterial identification

The department of laboratory of the bacteriology received 100 pus sample swab specimens from diabetic foot infection ulcers as part of the sample collecting process. Each sample was directly inoculated into a culture of a specific media, such as Mac.Conkey or Mannitol, and then incubated at 37°C for 18 to 24 hours [8].

It analyzed the colony characteristics under a microscope after gram-staining and performing a biochemical test to determine the type of bacteria present. Last but not least, identification was carried out automatically using the G-ve I. D cards and the V. ITEK-2 compact approach.

Antibiotic Susceptibility Test of K. pneumoniae

K. pneumaniae isolates were tested for antibiotic susceptibility using the disc diffusion method, as per. Disks were put after Mueller-Hinton plates had been inoculated with a 0.5 McFarland standard suspension of K. pneumoniae organisms. After overnight incubation, zones of growth inhibition were measured in millimeters. 9) The antibiotics ticarcillin-tazobactam, piperacillin-tazobactam, colistin, trimethoprim-sulfamethoxazole, cefepime, aztreonam, gentamicin, ceftazidime, meropenem, impenem, levofloxacin, cip

DNA Extraction of bacteria

By use (Genomic DNA promaga Kit).

Molecular Identification

The P.C.R assay was used to identify K. pneumoniae as shown in table by identifying the blashv gene (2). These primers were created by the Canadian business Alpha DNA, as shown in the table (1). 1% agarose gel electrophoresis is used to magnify results in order to determine the size of the PCR. The gel was stained for roughly 1.5 hours at 80 volts using 4 mL of ethidium bromide (10 mg/mL;Sigma, USA). The Mwut. of amplified products was measured using a 100bp ladder (Bionaer, Korea).

Table (1): Primers utilized in this study.

Primer	sequence	Amplicon size
shv	F: GAGCGAAAGATCCACTATCG R: GGTATCCCGCAGATAAATCA	525

Table (2): PCR program of shv primer that apply in the thermocycler

Gene	Primary denaturation	No.of cycles	Denaturation	Annealing	Extension	Final extension
shv	96 Cє at 4min.	30	96 Cє at 1min.	55 Cє at 1 min.	72 Cє at 1min.	72Cє at 10 min.

Results and Discussion

In this study a whole of (100) clinical specimen was together of patients suffering for diabetic foot. Patients of both sexes who are seen at various hospitals in the province of Al-Najaf range in age from (33-65) years. The findings showed that there were 26 (26%%) more females than males in this group. increased frequency of men compared to women, similar to the outcome of [10]. They showed that men made up 72.2% of the population, and (Bansal et al., 2008) they noticed that men were more common than women (78.64% vs. 21.36%). Additionally, patients in the hospitals in and near Coimbatore showed an increased ratio of men to women. 90 female and 180 male diabetes patients total suffered from foot infections. The patients' ages ranged from 36 to 75 years, with a male to female ratio of roughly 2:1. So few patients had to have their legs amputated. the research that was done in Chennai.

Their mobility compared to females [12], illustrate that wounds healing in females more better than in males, this may be due to hormonal differences and explain that increase estrogen receptor in female increase healing wound which act as endogenous enhancers of healing process while increase the level of androgen was considered harmful for wound healing in male increased the level of androgen species decrease repair of dermis.

A total number of 100 swabs about.The results revealed that 80 (80) % specimens gave up a positive culture and the other 20 (20)% were negative culture the wounds weren't infected at the time of the study or the antibiotics were effective, or the outcome of negative growth could be due to the other infectious agents, anaerobic bacteria, fungi, and viruses [13], or both [14]. A culture analysis based on morphological and biochemical tests revealed a high incidence of Gram-negative bacteria.

The results showed that G-ve bacteria revealed a high rate 69(69%) , 26(32.5%) K.pneumonia , 19 (23.75%) belonged to E.coli followed by p. aeruginosa 10 (12.5%) then proteus mirabillis 12 (15%) and proteus vulgaris 2(2.5%) as figure(1) only 8 (10%) isolates belong to S. aureus followed by S.epidermidis 3 (3.75%).

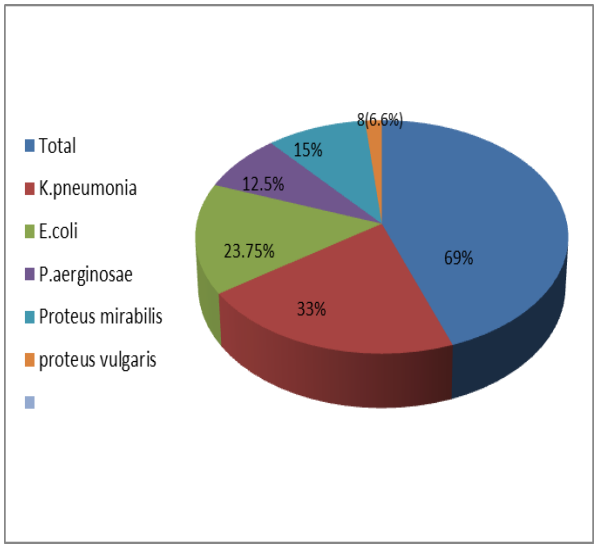

Figure (1): The percentage prevalence of bacteria

Among bacterial isolates, K. pneumonia 26(32.5) were isolated and diagnosed from clinical specimens of diabetic foot ulcer and detect the antibacterial resistance to some antibacterial agent phenotypically. this result was similar with result of [15] and [16],[17] found that (9%) of isolates were Klebsiella pneumonia. Klebisella spp, produce a mucoid lactose fermented colony on MacConkey agar and give indole negative, citrate positive non motile and A/A on TSI as table (3).

Table (3): biochemical test of K.pneumoniae

Tests / Type of bacteria	Oxidase	Motility	Indole	VP	MR	Citrate	Urease	TSI
Klebsella spp	-	-	-	+	-	+	+	A/A

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was conducted for (26.5) of K.pneumoniae isolates using disc diffusion way.

The resistance rate that show in (Figure 4-8 and table 4-3) of K. peumoniae isolates to Ticarcillin(88.8%) and Piperacillin-tazobactam (88.8%) were all high. The moderate resistance rate was observed for Trimethoprim-sulfamethoxazole (44.4%). The lowest resistance rate was observed for Cefepime (33.3%), Aztreonam (22.2%), Gentamicin (22.2%), Ceftazidime (11.1%), Meropenem (0%) and Impenem (0%), Levofloxacin (0%), Ciprofloxacin (0%), Amikacin (0%) and Tobramycin (0%). This result agreement with ((18)) who found that (0%) resistance to Amikacin. and agreement with ((19)) who found that (0%) resistance to Meropenem. Also this result near to ((20)) who found that (83%, 62%, 61%,28%, 57%, 89% and 56%) resistance to Gentamicin, Ciprofloxacin, Aztreonam, Piperacillin-tazobactam, Cefepime, Ceftazidime, and Impenem,. Also, this result disagreement with (21) who found that (40%) resistance to Tobramycin.

Detection of some virulence factors for K.pneumoniae

Phenotype detection of Biofilm Production

(TCP) Tissue culture plate was utilized to detection the biofilm manufacture of bacterial isolates, the quantefication result of biofilm formation by microtiter plate method revealed that The results confirmed that 20(76.9%) of K. pneumonia. The present study was demonstrated 15(75%) of isolates were strong to biofilm formation ,4(20%),1(5%) moderate and 0 weak by microtitier plate as show table (4). K. pneumaniae is capable to form biofilms, that is, aggregates in that cells embedded by a self-produced matrix of extracellular polymeric material abide to each another and/or to a surface [22],the utmost clinically important, K. pneumaniae biofilms are these formed on the internal surfaces of catheters and additional indwelling devices The colonization of the pulmonary, gastrointestinal, and urinary tracts as well as the growth of invasive infections, particularly in patients with impaired immune systems, may be facilitated by K. pneumaniae biofilms. The kind 3 fimbriae and the CP are the most important surface structures that were involved in the formation bacteria are both TEM and SHV process. While CPs ultimately influence cell to cell communication and biofilm development, fimbriae mediate stable adhesion.

Table (4) Classification of bacterial adherence and biofilm formation via tissue culture plate way(TCP)

Mean of O.D value at 630nm	Biofilm formation
< 0.120	Non
0.120- 0.240	Moderate
>0.240	High

Molecular Detection of shv gene of K. pneumaniae

According to the data in figure 2, 24 (92%) of the K. pneumaniae isolates had the shv gene identified;this result is consistent with finding [23]. All isolates were tested for the presence of the shv gene, including non-EsBL producers. The findings revealed that 60 [93.7%] of the total tested isolates carried the Shv gene. The purpose of this study was to determine the prevalence of K. pneumoniae that produces E.SBLs and the existence of SHV genes among isolates that do so. According to another study's findings, E. coli and K. pneumoniae that produce E.SBL are very common (15.62% and 20%, respectively), and 68.5% of these isolated

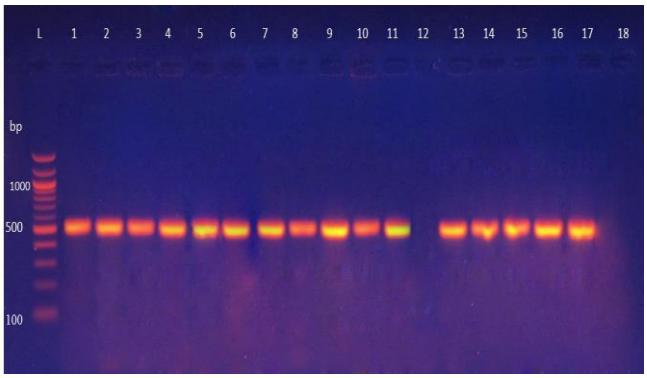

Figure (2): Using the primer shv gene with product 525 bp, P.C.R products from extracted entire DNA of K.pneumoniae were electrophoresed on agarose gels stained with ethidium bromide. At 70 volts for 1.5–2 hours, the electrophoresis was carried out. Lane (L), a DNA molecular size marker (1000 bp ladder). Lanes 1 through 18, excluding 12 and 18, display favorable results for the shv gene.

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