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MOLECULAR DETECTION OF SHV GENE PRODUCING KLEBSIELLA SPECIES IN STOOL SAMPLES COLLECTED FROM A TERTIARY HEALTH FACILITY

Pughikumo D. T.[1]*, Alade T.[2], Eromosele P. O.[2] and Pughikumo O. C.[3]

[1]Department of Human Physiology, Niger Delta University.
[2]Med. Lab. Sciences Department, Niger Delta University.
[3]Hematology unit, Niger Delta University.

***Corresponding Author: Pughikumo D. T.**
Department of Human Physiology, Niger Delta University.

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ABSTRACT
Bacterial resistance to antibiotics has been a major source of public health concern. Klebsiella pneumonia an important health pathogen is highly resistant to a wide range of antimicrobials and antibiotics. Investigation of beta-lactamase (extended-spectrum) - ESBL encoding-resistant gene, SHV in Klebsiella pneumonia isolated from clinical samples at a tertiary health facility, using standard Bacteriological technique. From fifty samples of clinical isolates used, thirty-three (33) were positive in this study for Klebsiella pneumonia, E. coli, Proteus mirabilis. 17 isolates were from female and 16 from males. 10 (100%) of the 33 samples were Klebsiella pneumonia isolates. Klebsiella pneumonia showed indole negative, citrate positive, it does not ferment lactose but ferment glucose, does not produce hydrogen sulphide but produces gas on Kliger Iron Agar (KIA). Amplification of SHV gene was done using PCR (Polymerase Chain Reaction) technique. The age base profiling of bacterial isolates showed that Klebsiella pneumonia was highest (80%) among 11 to 30 years. Antimicrobial susceptibility test was performed using disk diffusion method. The susceptibility pattern in the study revealed that Klebsiella pneumonia exhibited the highest susceptibility of 7(70%) for Reflacine, Streptomycin, Tarivid. 6 (60%%) for Ciprofloxacin and Gentamycin, and moderate susceptibility of 4(40%) for Sceptrin, 3(30%) Amplicin, Augmentin, and Nalidixic Acid. 2(20%) for Ceporex, which showed a high resistance to Klebsiella pneumonia. Identification of the gene that encodes plasmid transfer of SHV gene was done using multiplex PCR technique. From the results, accurate identification of resistant Gram-negative bacteria such as Klebsiella pneumonia and detection of its susceptibility to common antibiotics might enhance treatment and control of resistant nosocomial infections causing Klebsiella pneumoniae.

KEYWORDS: Klebsiella pneumonia, SHV, antibiotics, resistant gene, ESBL.

INTRODUCTION
Gram-negative bacterium - Klebsiella poses a significant health concern associated with majority of human infections. It thrives partly because of its ability to survive anaerobe and aerobe environments (Stojowska and Krawczyk, 2016; Han et al., 2015).

Klebsiella pneumonia is most common amongst three species in the genus Klebsiella. Its other species also exhibit high resistant traits to antibiotics (Goossens et al, 2015).

Its numerously virulence resisitance and strains requires innovative clinical managing approach; which also sets it as possible vaccine candidate (Amit et al., 2015).

The emergence of multidrug-resistant strains of Gram-negative klebsiella species is an urgent global threat. The World Health Organization has listed Klebsiella pneumonia alone of the global priority pathogens in critical need of next-generation antibiotics. Compared to other Gram-negative pathogens, Pneumonia accumulates a greatest diversity of antimicrobial-resistant genes at a higher frequency. The evolution of a hypervirulent phenotype of K pneumonia is yet another concern. It has a broad ecological distribution affecting humans, agricultural animals, plants and aquatic animals. (Marques et al, 2019).

However, Klebsiella pneumoniae and its other specie - oxytoca have agricultural relevance, (Goossens et al., 2015). And nominal bacterial testing is less recommended than molecular methods for accuracy, despite cost and technical demands (Tamara, 2017; Han *et al*, 2015; Kishibe *et al.*,2016).

This study focused on investigation of SHV gene in Klebsiella collected from stool sample, with specific

objectives of isolating and identifying Klebsiella species, extraction of the genomic DNA, amplification of 16srRNA gene and SHV gene in Klebsiella Species; perhaps this could contribute to therapeutic science linked with it (Shobha et al., 2019).

MATERIALS AND METHOD

Fifty (50) samples were obtained including urine and stool from tertiary health facility, o

Bacterial isolation involved stool samples being cultured into MacConkey agar respectively, the cultured plate was incubated in the incubator at 370 °C for 24hrs. The morphology of the bacterial colonies; size, pigmentation, surface and degree of opacity were macroscopically observed and recorded.

Gram staining was achieved by making smear on clean grease free glass slide, which emulsifies and dried, then subjected to Bunsen flame. Smear and slide were processed by protocol, and immersion oil dropped on the smear to view with X 100 oil immersion lens microscope.

Citrate Utilization and Indole tests were performed by standard procedure. Colonies were tested for their ability to ferment dextrose and lactose, as hydrogen sulphide producer, and for gas production by using a straight sterilised wire loop to collect the test organism from the peptone broth. It was stabbed on the butt of the test tube containing the Klingler iron agar and the slant was streaked. Following incubation, media was observed for hydrogen sulphide production and gas production.

Mortality test

The subcultures colonies of the organism in peptone broth were emulsified on microscope, cover slipped then mounted on microscope to view mortality of the organism.

Susceptibility testing

The antibiotic susceptibility testing of the Escherichia coli isolates was carried out by agar diffusion method using commercially available discs to determine the drug sensitivity and resistant Atem of the isolates. Susceptibility to an antimicrobial agent was marked by a clear zone of inhibition which was absent in the case of resistance.

Molecular methods

DNA extraction and quantification, 16SrRNA and SHV genes amplification were meticulously performed following standard protocol (Stojowska & Beata, 2016; Tamara, 2017).

RESULTS

Table1 (4.1): Shows the biochemical reaction and sugar fermentation of E. coli, Klebsiella and Proteus mirabilis.

Table 4.1: Biochemical reaction of the isolates

The table below shows the biochemical reaction of isolated organism using the various biochemical reagents.

ORGANISMS	INDOLE	CITRATE	H2S	GAS	SLANT	BUTT
E.Coli Species	+	-	-	+	Yellow	Yellow
Klebsiella Species.	-	+	-	+	red	Yellow
Proteus Species.	-	+	+	+	red	yellow

Key

:Negative (-),

Positive (+)

Table 2: Shows the distribution of patient based on their age and gender. The male were 17 (51.52%), and female 16 (48.48%).

Table 2: Demographic distribution of Subjects by Age and gender.

Age Range	Female	Male	Total
0-10	-	-	-
11-20	5	5	10
21-30	5	6	11
31-40	1	5	6
>40	6	-	6
TOTAL	17	16	33

Table 3: Shows the distribution of pathogen by age. Escherichia coli 9 (99.9%), Klebsiella pneumonia10 (100%) and Propteus mirabilis 1(100%).

Table 3: Distribution of bacteria Isolate by age

Age (years)	*Escherichia coli*	*Klebsiella pneumonia*	*Proteus mirabilis*	Total
0-10	-	-	-	-
11-20	3 (33.3%)	4 (40%)	1 (100%)	8
21-30	4 (44.4%)	4 (40%)	-	8
31-40	2 (22.2%)	1 (10%)	-	3
>40	-	1 (10%)	-	1
TOTAL	9 (99.9%)	10 (100%)	1 (100%)	20

Table 4: Shows distribution of bacteria isolate in relation to male gender. Males that fall between age 21-30 had the highest occurrence of infection with E. coli and less than klebsiella infection . E. coli had a total of 4(44.4%) and klebsiella had a total of 7(70%).

Table 4: Distribution of bacteria by gender (%)

organisms	Age	Gender (F)	Gender (M)
E.coli	11-40	4(44.4%)	5(55.5%)
Klebsisella	11-40	7(70%)	3(30%)
Proteus	11-40	-	1(100%)
Total		11(55%)	9(45%)

Table 6: Shows the antimetrical susceptibility of isolates using Ciprofloxacin, Septrin, Amplicin, Ceporex, Tarivid, Nalidixic Acid, Reflacine, Gentamycin, Augmentin, Levofloxacin, Erythromycin, Chloramphenicol, Rifampicin.

Table 6: Susceptibility test of the isolate

RGANIS IS	CPX	SXT	S	PN	CEP	OFX	NA	PEF	CN	AU	LEV	E	CH	RD
coli spp	8 (88.8 %)	2 (22.2 %)	7 (77.7 %)	1 (11. 1 %)	2 (22.2 %)	6 (66.6 %)	1 (11.1 %)	6 (66.6 %)	7 (77.7 %)	5 (55.5 %)	1 (11.1 %)	1 (11.1%)	2 (22.2%)	-
ebsiella spp	6 (60%)	4 (40%)	7 (70%)	3 (30 %)	2 (20%)	7 (70%)	3 (30%)	7 (70%)	6 (60%)	3 (30%)	-	-	-	-
roteus spp	1 (100 %)	-	1 (100 %)	-	-	-	-	-	-	-	1 (100%)	1 (100%)	1 (100%)	1 (100%)

KEY:

CPX: Ciprofloxacin, SXT: Septrin, S: Streptomycin, PN: Ampicilin, CEP: Ceporex, OFX: Tarivid, NA: NalidIxic Acid, PEF: Reflacine, CN: Gentamycin, AU: Aug-mentin, LEV: Levofloxacin, E: Erythromycin, CH: Chloramphenicol, RD: Rifampicin ()

Table 7: Shows the comparison of phenotypic and genotypic identification of selected isolates.

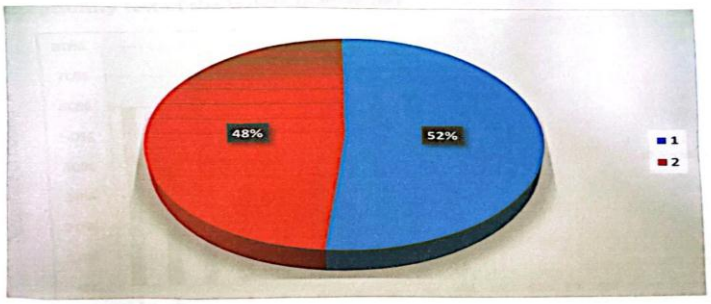
Table 7: comparison of phenotypic and genotypic identification

bacteria isolates from each of the organism identified phenotypically were randomly selected for sequencing and the phenotypic and genotypic comparism is shown on the table below;

ISOLATES	PHENOTYPIC	GENOTYPIC
Isolate 1	*Klebsiella*	*Klebsiella pneumoniae*
Isolate2	*Klebsiella*	*Klebsiella pneumoniae*
Isolate 3	*Klebsiella*	*Klebsiella pneumoniae*

Phenotypic identification of bacteria organism was done and were randomly selected for sequencing, after sequencing, three isolates were seen genotypically indicating *Klebsiella pneumonia.*

Chart 1: Demographic distribution based on gender

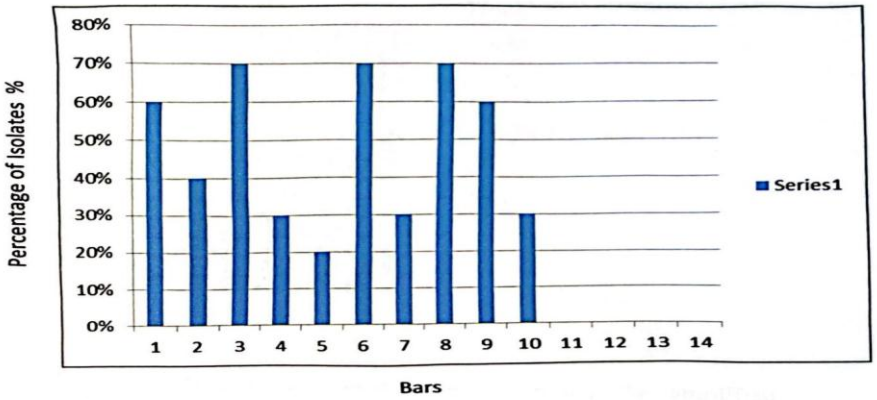


KEY

1 FEMALE

2 MALE

Chart 3: Susceptibility test of the isolate (*Klebsiella*)



KEYS

Where bar 1 represents CPX at 60%

Bar 2 represents STX at 40%

Bar 3 represents S at 70%

Bar 4 represents PN at 30%

Bar 5 represents CEP at 20%

Bar 6 represents OFX at 70%

Bar 7 represents NA at 30%

Bar 8 represents PEF at 70%

Bar 9 represents CN at 60%

Bar 10 represents AU at 30%

Bars 11,12,13 and 14 shows to be at 0%.

a mass ruler high range DNA ladder.

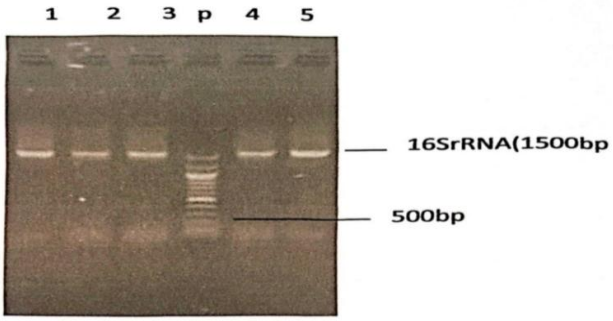


Plate 1: Agarose gel electrophoresis showing the amplified 16SrRNA fragment. Lanes 1-10 represent the 16srRNA at 1500bp while lane L represents the 100bp molecular ladder

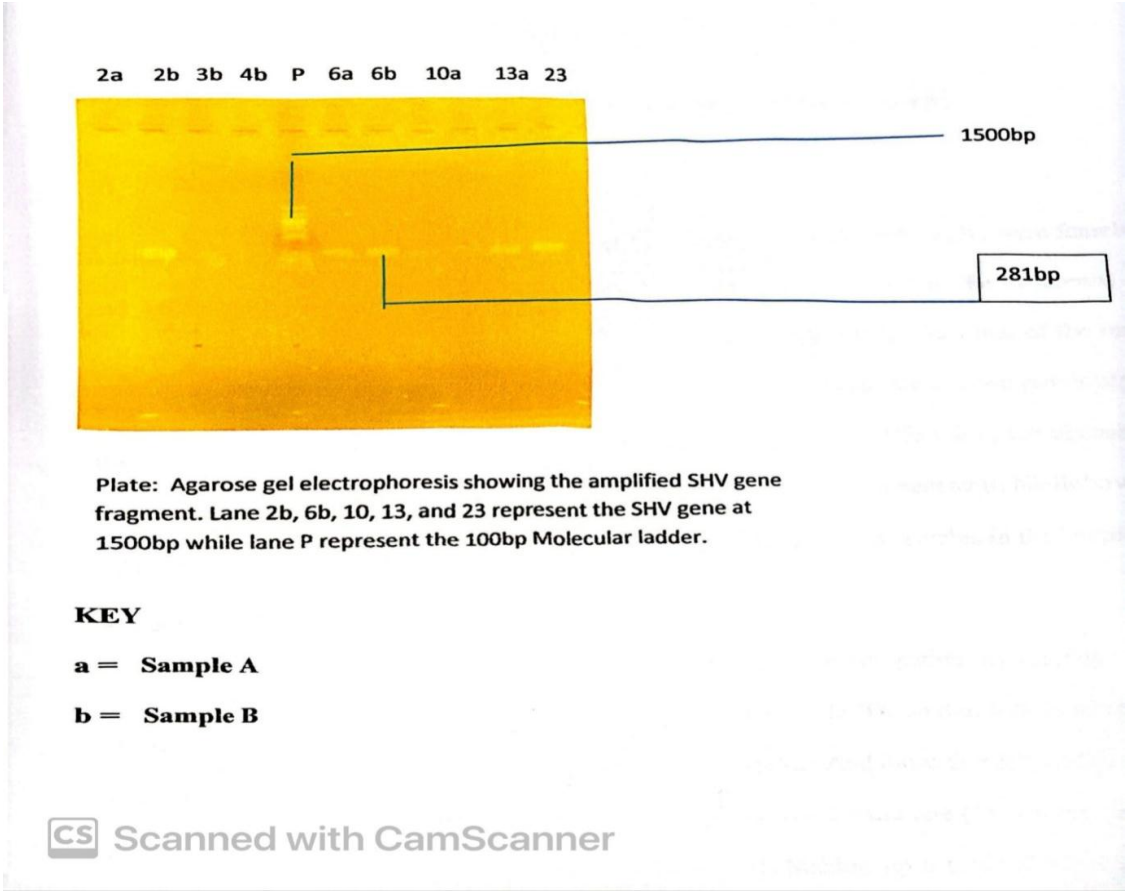


Plate: Agarose gel electrophoresis showing the amplified SHV gene fragment. Lane 2b, 6b, 10, 13, and 23 represent the SHV gene at 1500bp while lane P represent the 100bp Molecular ladder.

KEY

a = Sample A

b = Sample B

DISCUSSION

A total of 50 clinical samples were isolated wherein 33 were positive, among which were 17(51.52%) females and 16(48.48%) males. Perhaps the high occurrence in females may be due to difference in urogenital anatomical structure; as urethra in females are shorter than that of the male which may allow easy access of microorganisms into the body. Also, close proximity of the urethra and vagina to the anal canal, makes them prone. During cleanup, following defecation, organisms can be introduced into the vagina leading to infection which corroborates previous report (Li et al, 2016; Liu & Guo , 2020).

The demographic distribution of Klebsiella pneumonia in the individuals by age reveals that isolated bacteria of involved individuals in this research showed klebsiella spp is most prevalent in the age group 11-30.

The biochemical reaction that was carried out on the thirty-three isolates which shows the phenotypic analysis of Escherichia coli was indole positive, citrate negative and lactose fermentation positive on KIA and gas production. Phenotypic characterization of klebsiella specie showed indole negative, citrate positive, ferments glucose, but does not ferment lactose and does not produce hydrogen sulphide, rather produces gas on KIA. And the phenotypic characterization of Proteus specie was indole negative, citrate positive, presence of swarming on plate and does not ferment lactose but utilizes glucose on KIA.

The overall incidence of antibiotics resistance of Klebsiella pneumonia was high in this study. The susceptibility pattern for Klebsiella spp isolates revealed that Klebsiella pneumonia exhibited the highest susceptibility of 7(70%) for Reflacine, Streptomycin, Tarivid. 6(60%) for Ciprofloxacin and Gentamycin, then moderate susceptibility of 4(40%) for Septrin. And 3(30%) for amplicin, augmentin, with Nalidixic Acid. Meanwhile, it was 2(20%) for Ceporex, which showed a high resistance to Klebsiella pneumonia.

The comparison of phenotypic and genotypic identification of selected isolates shows that three were confirmed to be Klebsiella pneumoniae both phenotypically and genotypically. But genotypic characterization has more advantage over the phenotypic because it is fast, accurate, more specific and precise.

Meanwhile, prevalence of SHV gene as shown by 1 % agarose gel electrophoresis, indicated by the positive band with [P representing the 1000 base pair Quick-Molecular DNA Ladder infers that patients within these areas are prone to infections caused by Klebsiella pneumonia. This high rate of resistance may be attributed to misuse of antibiotics, readily administered drugs in hospitals and the presence of drug resisting mechanism

such as transfer of plasmid genes, and presence of drug resistant gene.

CONCLUSION

In conclusion, further investigations unravelling SHV gene is worthwhile, and characterizing bacteria genotypically using the16srRNA gene sequencing and the amplification of SHV gene is more reliable than phenotypic characterization in minimising errors from misinterpretation of biochemical test.

REFERENCES

1. Amit, S., Mishali, H., Kotlovsky, T., Schwaber, M.J., and Carmeli, V. Bloodstream infections among carriers of carbapenem-resistant Klebsiella pneumoniae: etiology, incidence and predictors. Clinical Microbiology Infection, 2015; 21(1): 30-4.
2. Goossens, H., Izdebski, R., Fiett, J., Urbanowicz, P., Baraniak, A., Derde, L.P., Bonten, M.J., Carmeli, Y., Hryniewicz, W.,Brun, B. C.,Brisse, S.,and Gniadkowski, M. Phylogenetic lineages, clones and β-lactamases in an international collection of Klebsiellaoxytoca isolates non-susceptible to expanded-spectrum cephalosporins. Journal of Antimicrobial Chemotherapy, 2015; 70(12): 3230-3237.
3. Han, S., Lee, S., Jeong, D., and Kang, J. Aminoglycoside therapy for childhood urinary tract infection due to extended-spectrum β-lactamase producing Escherichia colior Klebsiella pneumoniae. BMC Infectious Diseases, 2015; 15: 414.
4. Kishibe, S.,Okubo, Y.,Morino, S.,Hirotaki, S.,Tame,T.,Aoki, K.,Ishii, Y., Ota,N., Shimoomura,S., Sakakibara, H.,Terakawa,T., and Hirokoshi, Y. Paediatric hypervirulent Klebsiella pneumoniae septic arthritis. Paediatrics International, 2016; 58: 382-385.
5. Kovarik, P.,Castiglia, V., and Ivin,M. Type I interferons in bacterial infections:Abalancing act Frontiers in Immunology, 2016; 7: 652.
6. Lachmandas, E.,Boutens, L.,and Ratter, J.M. Microbial stimulation of different toll-like receptor signalling pathways induces diverse metabolic programmes in human monocytes. Nature Microbiology, 2016; 2: 16246.
7. Li, Y., Oosting, M., and Deelen, P. Inter-individual variability and genetic influences oncytokine responses to bacteria and fungi. Nature Medicine, 2016; 22: 952-60.
8. Liu, C. and Guo, J. Characteristics of ventilator-associated pneumonia due tohypervirulent Klebsiella pneumoniae genotype in genetic background for the elderly intwo tertiary hospitals in China Antimicrobial Resistant Infection and Control, 2020; 7: 95.
9. Marques, C., Belas, A., Aboim, C., Cavaco, S. P., Trigueiro, G., and Gama, L. T. Evidenceof sharing of Klebsiella pneumoniae strains between healthy companion animals andcohabiting humans. Journal of Clinical Microbiology, 2019; 57(6).
10. Marques, C., Menezes, J., Belas, A., Aboim, C., Cavaco, S. P., and Trigueiro, G. Klebsiellapneumoniae causing urinary tract infections in companion animals and humans: populationstructure, antimicrobial resistance and virulence genes. Journal of Antimicrobial Chemother, 2019; 74(3): 594-602.
11. Stojowska, S. K.,and Beata, K. B. A new assay for the simultaneous identification and differentiation of Klebsiella oxytoca strains. Applied Microbiology and Biotechnology, 2016; 100(23): 10115-10123.
12. Tamara, H. Z. Molecular discrimination of Klebsiella oxytoca using polymerase chainreaction targeted polygalacturonase (pehX) Gene. International Journal of Current Microbiology and Applied Sciences, 2017; 6(6): 2092-2098.