www.ajbrui.net

Afr. J. Biomed. Res. Vol.17 (September, 2014); 203- 207

Full Length Research Paper

Haemolysin and Serum Resistance Profiles of Bacteria Isolates from Blood Culture

Egbe C.A*[1] and Enabulele O.I[2]

[1]*Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City. Edo State, Nigeria.*
[2]*Department of Microbiology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria*

ABSTRACT

Haemolysin production and serum resistance ability of bacteria isolates from blood culture were investigated using standard microbiological technique. *Enterobactor* species were the bacteria isolates with the highest prevalence of β–haemolysin production while *klebsiella* species were the least. No strain of *Citrobacter freundii, Proteus vulgaris, Alcaligenes* species and coagulase negative *Staphylococcus* produced haemolysis. No bacteria species was sensitive to the bactericidal action of normal human serum. There was no association between haemolysin production and resistance to ciprofloxacin, ceftriaxone and ceftazidime. Conclusively, bacteria agents of septicemia are very virulence and urgent treatment is advocated to stem the tile of associated sequelae.

Key words: Hemolysin production, serum resistance, bacterial isolates, blood culture

INTRODUCTION

Haemolysin is among the important virulent factors that are well studied especially in the organism *Escherichia coli* (Moxley *et al.*, 1998), and it has been reported that haemolysin increases the virulence of *Escherichia coli* in the blood stream and peritoneal cavity (Emody *et al*., 1980). Haemolysin has been suggested to increased virulence by increasing the availability of iron mediating toxic effect on leucocytes and other nucleated cells potentiating the effect on endotoxin, and mediating serum resistance (Welch *et al.,* 1981; Opal *et al.,* 1990; Siegfried *et al.,* 1995). Surprisingly some authors have linked loss of haemolysin producing ability to quinolones and flouroquinolones resistance (Martinez-Martinez *et al.,* 1999; Drews *et al.,* 2005). However, the exact mechanism is not known (Drews *et al.,* 2005).

Serum resistance is an important determinant of the pathogenicity of Gram negative bacteria. (Leying *et al.,* 1990). The bactericidal activity of normal serum is generally considered to be an important host defence against bacterial infections (Montenegro *et al.,* 1995). As a consequence, resistance to serum killing is regarded as an important virulence property of invasive bacteria (Montenegro *et al.,* 1985). It has been reported that bacteria isolated from the blood of patients with bacteremia/septicaemia are more likely to be resistant to serum than those isolated from faces of healthy individuals (Roantree and Rantz, 1990; Vosti and Randale, 1990). Serum resistance ability has been attributed to several distinct components of bacteria surface which include O-side chains of lipopolysaccharides, capsular antigen and surface protein (Montenegro *et al*., 1985; Fang *et al.,* 2004). Serum resistance of Gram negative is controlled by 2–genes–VacJ and Yrb genes that encoded for synthesis of the Oligosaccharides component of bacteria outer membrane and the ABC transporter responsible for the retrograde trafficking of phospholipids from outer to inner leaflet of the cell envelope (Nakamura *et al.,* 2011). To our knowledge, no study within our locality has examined the serum resistance and haemolysin profile of bacteria isolates form blood culture. Hence this study was conducted to determine the haemolysin and serum resistance profile of bacteria isolates from blood culture. The association between haemolysin production and bacteria resistance to ciprofloxacin, ceftazidime and ceftriaxone was also assessed.

MATERIALS AND METHODS

Bacterial isolates:

A total of 104 consecutive and non-repetitive bacteria isolates from blood culture were used for this study. The

isolates were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital, Benin city, Nigeria. The bacteria isolates includes. *Klebsiella* species (36), *Enterobacter* species (8), *Escherichia coli* (9), *Citrobacter* species (2), *Proteus vulgaris* (5), *Proteus mirabilis* (10), *Acinetobacter* species (7), *Providencia* species (1), *Alcaligenes* species (2), *Staphylococcus aureus* (20), coagulase negative *Staphylococci* (3), *Streptococcus pneumoniae* (1). They were identified using standard techniques (Barrow and Fethham 2003).

Haemolysin production:
Haemolysin production was detected using the method described by Martinez–Martinez *et al.* (1999). All bacterial isolates were grown on 5% sheep blood agar at 37^0C for 24 hours. The presence of a clear zone around the colonies was taken as positive for haemolysin production.

Determination of serum resistance:
The serum resistance assay was performed by the method described by Kumar and Mathur (1997). Pooled normal serum was obtained from 12 apparently healthy individuals. The test organisms were grown in separate nutrient broth at 37^0C overnight. The turbidity of the overnight broth culture was matched with McFarland standard (0.5 Brown's opacity tubes) and then adjusted to a count of 10^4 cfu/ml in 5ml fresh nutrient broth and incubated at 37^0C for 2hours. Cultures were centrifuged at 1500g for 5min and the deposit resuspended in 5ml sterile phosphate buffered saline (PBS). Equal volume (0.5ml each) of pooled normal human serum and bacteria suspension were mixed and incubated in a water bath at 37^0C. Viable count was performed on the pooled normal human sera and the bacteria suspension mixture at 0 and 3 hours using the surface spreading method. If the viable count dropped to less than 1% of the initial value (comparing counts at 0 and 3 hours) the isolates was regarded as sensitive. If more than 90% of the organism survived after 3 hours the isolate was said to be resistant. Counts between 1 and 90%, the isolate was termed intermediate.

Disc susceptibility test:
Disc susceptibility test was performed according to the British Standard for Antimicrobial chemotherapy (BSAC) method (Andrews, 2009).

Statistical analysis:
The data were analyzed using Fisher's exact test and odd ratio using the statistical software INSTAT®.

RESULTS

Citrobacter freundii, Proteus vulgaris, Providencia species, *Alcaligenes* species and coagulase negative *staphylococci* did not produce haemolysin while *Enterobacter* species, *Acinetobacter* species, *Escherichia coli, Proteus mirabilis, Staphylococcus aureus* and *Klebsiella* species produced haemolysin in descending order of prevalence. No bacterial isolates recovered were susceptible to the bactericidal action of normal human serum as majority of the isolates were resistant to the bactericidal action of serum with few genera of bacteria showing intermediate resistant (Table 1). There were no association between haemolysin production and ciprofloxacin, ceftazidine and ceftriaxone resistance as the observed differences were statistically insignificant (Tables 2, 3, 4).

Table 1:
Haemolysin production and serum resistance profiles of bacterial agents of septicaemia

organisms	Number tested	Number positive for haemolysin (%)	Serum resistance (%)	
			Intermediate	**Resistance**
Klebsiella spp	36	9 (25.00)	7 (19.44)	29 (80.56)
Enterobacter spp	8	6 (75.00)	3 (37.50)	5 (62.50)
Escherichia coli	9	5 (55.56)	0 (0.00)	9 (100.00)
Citrobacter freundii	2	0 (0.00)	0 (0.00)	2 (100.00)
Proteus vulgaris	5	0 (0.00)	0 (0.00)	5 (100.00)
Proteus mirabilis	10	4 (40.0)	0 (0.00)	10 (100.00)
Acinetobacter spp	7	5 (71.43)	0 (0.00)	7 (100.00)
Providencia spp	1	0 (0.00)	0 (0.00)	1 (100.00)
Alcaligenes spp	2	0 (0.00)	0 (0.00)	2 (100.00)
Staphylococcus aureus	20	6 (30.00)	40 (20.00)	16 (80.00)
Coagulase negative *staphylococcus*	3	0(0.00)	0 (0.00)	3 (100.00)
Streptococcus pneumoniae	1	0(0.00)	0 (0.00)	1 (100.00)

Table 2:
Association of ciprofloxacin resistance with haemolysin production

Organism	No. (%) isolates with haemolysin	OR	95CI	P-value
Klebsiella spp				
Resistant	1(11.1)			
Sensitive	8(88.9)	0.37	0.038,3.390	0.648
Enterobacter spp				
Resistant	3(50.0)			
Sensitive	3(50.0)	5.000	0.170,146.76	0.464
Escherichia coli				
Resistant	1(20.0)			
Sensitive	4(80.0)	0.25	0.013,4.732	0.524
Proteus mirabilis				
Resistant	0(0.0)			
Sensitive	4(100.0)	0.20	0.007,5.457	0.467
Acinetobacter spp				
Resistant	2(40.0)			
Sensitive	3(60.0)	3.571	0.114,111.80	1.000
Staphylococcus aureus				
Resistant	3(50.0)			
Sensitive	3(50.0)	0.400	0.055,2.888	0.613

OR = Odd ratio; CI confidence interval

Table 3:
Association of ceftazidime resistance with haemolysin production

Organism	No. (%) isolates with haemolysin	OR	95CI	P-value
Klebsiella spp				
Resistant	1(11.1)			
Sensitive	8(88.9)	0.213	0.023,1.959	0.223
Enterobacter spp				
Resistant	1(16.67)			
Sensitive	5(83.33)	0.039	0.001,1.253	0.048
Escherichia coli				
Resistant	0(0.00)			
Sensitive	5(100.00)	0.055	0.002,1863	0.107
Proteus mirabilis				
Resistant	0(0.0)			
Sensitive	4(100.0)	0.009	0.000,0.516	0.005
Acinetobacter spp				
Resistant	1(20.0)			
Sensitive	4(80.00	1.667	0.048,58.335	1.000
Staphylococcus aureus				
Resistant	2(33.33)			
Sensitive	4(66.67)	6.500	0.459,91.984	0.202

OR = Odd ratio; CI confidence interval

DISCUSSION

In this study, *Enterobacter species* (75%), Acinetobacter species (71.43%), Escherichia *coli* (55.56%), *Proteus mirabilis* (40%), *Staphylococcus aureus* (30%) and *Klebsiella* species (25%) isolates produced β–haemolysin. Haemolysin production has being linked with bacterial resistance to antibacterial agents as well as expression of other virulence factors (Martinez-Martinez *et al.*, 1999; Drews *et al.*, 2005). Therefore the isolates that produced β–haemolysin in this study maybe more virulent and result in more severe form of bacterial septicaemia.

Citrobacter freundii, *Proteus vulgaris*, *Providencia* species, *Alcaligenes* species, coagulase negative staphylococcus and *Streptococcus pneumonia* did not produce β–haemolysin.

Table 4:
Association of ceftriaxone resistance with haemolysin production

Organism	No. (%) isolates with haemolysin	OR	95CI	P-value
Klebsiella spp				
Resistant	0(0.00)			
Sensitive	9(100.00)	0.121	0.006,2.322	0.160
Enterobacter spp				
Resistant	2(33.33)	3.889	0.137,110.08	0.500
Sensitive	4(66.67)			
Escherichia coli				
Resistant	0(0.00)	0.152	0.004,5.188	0.375
Sensitive	5(100.00)			
Proteus mirabilis				
Resistant	0(0.0)			
Sensitive	4(100.0)	Not calculated		
Acinetobacter spp				
Resistant	1(20.00)	3.571	0.114,111.80	1.000
Sensitive	4(80.00)			
Staphylococcus aureus				
Resistant	1(16.67)	7.009	0.278,225.11	0.300
Sensitive	5(83.33)			

OR = Odd ratio; CI confidence interval

The finding that no strain of *Providencia* species produced haemolysin agrees with earlier report (Senior and Hughes, 1987). *Alcaligenes* species are known not to produce any virulence factor (Forbes *et al.,* 2002). This may explain the result with *Alcaligenes* species in this study. Majority of the isolates – *Escherichia coli*, *Acinetobacter* species, *Providencia* species, coagulase negative staphylococcus, *Streptococcus pneumoniae* (100% each), *Klebsiella* species (80.56%) and *Enterobacter* species (62.50%), were resistant to the bactericidal action of serum. The bactericidal activity of normal serum is generally considered to be an important host defense against bacterial infections (Montenegro *et al.,* 1985). As a consequence, resistance to serum killing is regarded as an important virulence property of invasive bacteria (Montenegro *et al.,* 1985)

It has been reported that bacteria isolated from the blood of patients with bacteremia/septicaemia are more likely to be resistant to serum than those isolated from faeces of healthy individuals (Roantree and Rantz, 1990; Vosti and Randale, 1990). This notion agrees with the findings in this study. Serum resistance ability has been attributed to several distinct components of bacteria surface which include O-side chains of lipopolysaccharides, capsular antigen and surface proteins (Montenegro *et al.,* 1985; Fang *et al.,* 2004).

A number of studies have linked haemolysin production to antibacterial resistance (Martinez-Martinez, 1999; Drews *et al.,* 2005). However, in this study there was no significant association between haemolysin production and resistant to the antibacterial agents used in this study. The difference between the findings in this study and other reports maybe in the number and type of isolates used. In the studies of Martinez-Martinez *et al.,* (1999) and Drews *et al.* (2005) they used only *Escherichia coli* and a large number of strains while various genera of bacterial were used in our study and the sample size of each genera was small.

In conclusion, some genera of bacteria isolated from blood culture produced haemolysin, while none were susceptible to human serum. Although no significant association was found between haemolysin production and antibacterial resistance, urgent treatment of bacterial septicaemia is advocated to stem the tide of sequelae associated with these virulence pathogens.

REFERENCES

Andrews, J.M. (2009). BSAC standardized disc susceptibility testing method (version 3) J. Antimicrob. Chemother. **53*:*** 713 – 728.

Barrow, G.I. and Feltham, R.K.A. (2003). Cowan and Steel's manual for the identification of medical bacteria (3rd End). Cambridge University Press, Cambridge.

Drews, S.J., Poutanen, S.M., Maszzulli, T., McGeel, A.J., Sarabina, A., Pong-Porter, S., Rzayev, Y., Willeyi, B., Green, K. and Low, D.E. (2005). Decreased prevalence of virulence factors among ciprofloxacin-resistant uropathogenic *Escherichia coli* isolates. J. Clin. Microbiol **43**(8)**:** 4218 – 4220.

Emody, T.P., Safonova, N.V., Kuch, B. and Golutva, N.K. (1980). Alpha-haemolysin an additive virulence factor in

Escherichia coli. Acta. Microbiol. Acad. Sci. Hung. **27:** 233 – 342.

Fang, C.T., Chiang, Y.P., Shun C.T. Cheng, S.C. and Wang. J.T. (2004). A novel virulence gene in *Klebsiella pneumonia* strains causing primary liver abscess and septic metastatic complications. J. Experiment. Med. **199**(5):697 – 705.

Forbes, B.A., Sahn, D.I. and Weissfield, A.S. (2002). Barley and Scott's Diagnostic Microbiology (11th Edn) Mosby Inc. St. Louis

Kumar, S. and Mathur, M.D. (1997). Sensitivity to the bactericidal effect of human serum of *Proteus* strains from clinical specimens. Indian J. Pathol Microbiol. **40:** 335 – 339.

Leying, H., Suerbaum, S., Kroll, H.P., Stal, D. and Opferkuch, N. (1990). The capsular polysaccharide is a major determinant of serum resistance in K-1-positive blood culture isolates of *Escherichia coli* Infect immun. **58**(1): 222 – 227.

Maztine-Martinez, L, Fernandez, F. and Perea, E.J. (1990). Relationship between haemolysin production and resistance to fluroquinolones among clinical isolates of *Escherichia coli.* J. Anitimicrob. Chemother. **45:** 277 – 279.

Montenegro, M.A., Ritter-Suermann, D., Timmis, J.K., Aguero, M.E., Cabello, F.C., Sanyal, S.C. and Timmis, K.N. (1985). TraTGene sequences, serum resistance and pathogenicity related factors in clinical isolates of *Escherichia coli* and other Gram negative bacteria. J .General Microbiol. **131:** 1511 – 1521.

Moxley, R.A., Berberon, E.M., Francis, D.E., Xing, J., Moayeri, M., Welch, R.A., Baker, D.R. and Barletta, R.G (1998). Pathogenicity of an enterotoxigenic *Escherichia coli* haemolysin (hly A) Mutant in Gnotobiotic piglets. *Am. Soc. Microbiol* ***68***(10): 5031 – 5035.

Nakamura, S., Schchepefor, M., Dalia, A.B., Clark, S.E., Murphy, T.F., Sethi, S., Gilsodorf, J.R., Smith, A.L. and Weiser, J.N. (2011). Molecular Bases of increased serum resistance among pulmonary isolates of non-type-able *Haemoplilus influence.* Plos Pathog. 7(1): e1001247. doi:10.1371/journal.ppat.1001247

Opal, S.M., Cross, A.S., Gemski, P. and Lyhte, L.W. (1990). Aerobactin and β–haemolysin as virulence determinants in *Escherichia coli* isolated from human blood, urine and stood. J. Infect. Dis. **161*:*** 794 – 796.

Roantree, R.J. and Rantz, L.A. (1990). A study of the relationship of the normal bactericidal activity of human serum to bacterial infection. *J. Clin. Invest.* ***39:*** 72 – 81.

Senior, B.W. and Hughes, C. (1987). Production and Properties of haemolysin from clinical isolates of Protease. J. Med. Microbiol. **24:** 17 – 25.

Siegfried, L., Kemtova, M., Janigova, V., Sasinka, M. and Takacova, V. (1995). Serum response of *Escherichia coli* strains causing dyspepsia and urinary tract infection: relation to alpha-haemolysin production and O type. Infect. Immun **63:** 4543 – 4545.

Vosti, K.L. and Randale, E. (1990). Sensitivity of serologically classified strains of *Escherichia coli* of human origin to the serum bactericidal system. Am. J. Med. Sci. **259:** 114 – 119.

Welchi, R.A., Dellingner, E.P., Minshew, B. and Falkow, E. (1981). Haemolysin contributes to virulence of extra intestinal *Escherichia coli* infections. Nature **294:** 665 – 667.