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Haemolysin and Serum Resistance Profiles of Bacteria Isolates from Blood Culture

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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 haemolysin. 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 tide 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 flouoroquinolones 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 Fetham 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°C 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°C 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 10⁴ cfu/ml in 5ml fresh nutrient broth and incubated at 37°C for 2 hours. 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°C. 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, ceftazidime 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
<i>Klebsiella</i> spp	36	9 (25.00)	7 (19.44)	29 (80.56)
<i>Enterobacter</i> spp	8	6 (75.00)	3 (37.50)	5 (62.50)
<i>Escherichia coli</i>	9	5 (55.56)	0 (0.00)	9 (100.00)
<i>Citrobacter freundii</i>	2	0 (0.00)	0 (0.00)	2 (100.00)
<i>Proteus vulgaris</i>	5	0 (0.00)	0 (0.00)	5 (100.00)
<i>Proteus mirabilis</i>	10	4 (40.0)	0 (0.00)	10 (100.00)
<i>Acinetobacter</i> spp	7	5 (71.43)	0 (0.00)	7 (100.00)
<i>Providencia</i> spp	1	0 (0.00)	0 (0.00)	1 (100.00)
<i>Alcaligenes</i> spp	2	0 (0.00)	0 (0.00)	2 (100.00)
<i>Staphylococcus aureus</i>	20	6 (30.00)	40 (20.00)	16 (80.00)
Coagulase negative <i>staphylococcus</i>	3	0(0.00)	0 (0.00)	3 (100.00)
<i>Streptococcus pneumoniae</i>	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
<i>Klebsiella</i> spp				
Resistant	1(11.1)			
Sensitive	8(88.9)	0.37	0.038,3.390	0.648
<i>Enterobacter</i> spp				
Resistant	3(50.0)			
Sensitive	3(50.0)	5.000	0.170,146.76	0.464
<i>Escherichia coli</i>				
Resistant	1(20.0)			
Sensitive	4(80.0)	0.25	0.013,4.732	0.524
<i>Proteus mirabilis</i>				
Resistant	0(0.0)			
Sensitive	4(100.0)	0.20	0.007,5.457	0.467
<i>Acinetobacter</i> spp				
Resistant	2(40.0)			
Sensitive	3(60.0)	3.571	0.114,111.80	1.000
<i>Staphylococcus aureus</i>				
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
<i>Klebsiella</i> spp				
Resistant	1(11.1)			
Sensitive	8(88.9)	0.213	0.023,1.959	0.223
<i>Enterobacter</i> spp				
Resistant	1(16.67)			
Sensitive	5(83.33)	0.039	0.001,1.253	0.048
<i>Escherichia coli</i>				
Resistant	0(0.00)			
Sensitive	5(100.00)	0.055	0.002,1863	0.107
<i>Proteus mirabilis</i>				
Resistant	0(0.0)			
Sensitive	4(100.0)	0.009	0.000,0.516	0.005
<i>Acinetobacter</i> spp				
Resistant	1(20.0)			
Sensitive	4(80.00)	1.667	0.048,58.335	1.000
<i>Staphylococcus aureus</i>				
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 been 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 may be more virulent and result in more severe form of bacterial septicemia.

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

Table 4:
Association of ceftriaxone resistance with haemolysin production

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

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