

INCIDENCE OF ENTERIC BACTERIA AND *STAPHYLOCOCCUS AUREUS* IN DAY CARE CENTERS IN AKWA IBOM STATE, NIGERIA

Alfred Young Itah and Agnes Edet Ben

Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Abstract. The incidence of enteric bacteria and *Staphylococcus aureus* in four Day Care Centers in Akwa Ibom State was studied using culture techniques. The percentage frequencies of the isolates from 124 samples were *Staphylococcus aureus* (33.9), *Escherichia coli* (19.0), *Klebsiella* sp (14.4), *Citrobacter* sp (12.5) and *Proteus mirabilis* (7.4). The sources of contamination were floors, chairs, skin, bed linen, door handles, fans, children's tables, walls, windows, ceiling, headmistress's table and chairs, drinking water and wash water. Cultures from Aunty Chimmy's Day Care and Nursery School, Eket and Ideal Day Care and Nursery School, Eket yielded more organisms than those from Trinity International Nursery School, Ikot Ekpene and Adiaha Obong Day Care Center in Uyo. The results revealed the insanitary conditions in these day care centers. The enforcement of an effective public health enlightenment program is advocated in order to attract sufficient attention of the proprietors of these establishments to the role of fomites as reservoirs of pathogenic microorganisms.

INTRODUCTION

The Enterobacteriaceae (enterics) are gram-negative rod-shaped bacteria that include members of the genera *Escherichia*, *Enterobacter*, *Citrobacter*, *Salmonella*, *Serratia*, *Klebsiella* and *Shigella*. Others are *Proteus*, *Erwinia*, *Yersinia*, *Hafnia*, *Vibrio* and *Providencia*. The enterics are known to cause diseases such as typhoid and paratyphoid fever, pneumonia, cholera, wound infections, meningitis and food borne diseases in man. *Escherichia coli* has three pathogenic strains, namely *Enteropathogenic E. coli* (EPEC), *Enterotoxigenic E. coli* (ETEC), and *Enteroinvasive E. coli* (EIEC) with their pathophysiology well documented (Snydman, 1989; Pelczar *et al*, 2000). According to Holt *et al* (1994), the Enterobacteriaceae are responsible for about 50% of nosocomial infections most frequently caused by *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Providencia* and *Serratia marcescens*. *E. coli* is one of the most frequent enteric bacteria causing diseases in infants (Snydman, 1989). The disease is characterized by severe diarrhea and dehydration caused by enterotoxigenic and enteropathogenic strains. In Nigeria, Anozie and Antai (1987) isolated strains of enteropathogenic

E. coli from infantile diarrheal stools in Port-Harcourt metropolis. The organism was identified as the etiologic agent of about 15% of the infantile diarrheal outbreak in a community. Enteric bacilli, especially *E. coli* and *Klebsiella*, have been implicated in infantile meningitis (Prescott *et al*, 2002). Itah (1991) in his studies on ileal loop reactive *Escherichia coli* serotypes isolated from infantile diarrheal stools in Calabar, Nigeria, reported 47.2% incidence of ileal loop reactive *E. coli* strains.

The pathogenicity and pathophysiology of *Staphylococcus aureus* have been well documented (Anozie and Antai, 1987; Itah and Opara, 1997; Itah, 1999). Large numbers of *S. aureus* are disseminated from wounds, burns, nostrils and the skin into the environment. Equipment such as trolleys, beds, benches, chairs in homes and hospitals are often sources of contamination and infection (Raphael *et al*, 1976; Itah *et al*, 1998). Enteric pathogens, principally *Vibrio cholerae*, *Salmonella typhi* and *Salmonella paratyphi*, *Escherichia coli* from the soil or air can contaminate fomites which then become a source of contamination of children (Parker, 1978).

This paper aims at determining the hygienic status of day care centers in Akwa Ibom State with a view to offering useful advice to the managers of such centers and public health authorities.

Correspondence: Alfred Young Itah, Department of Microbiology, University of Uyo, PMB 1017, Uyo, Akwa Ibom State, Nigeria.

MATERIALS AND METHODS

The study area

The four day care centers under study were Aunty Chimmy's Day Care and Nursery School, Eket (DC I), Ideal Day Care and Nursery School, Eket (DC II), Trinity International Nursery School, Ikot Ekpene (DC III), and Adiaha Obong Day Care Center, Uyo (DC IV).

Sources and collection of samples

Samples were collected from door handles, floors, window blinds, windows, tables, walls, fans, the headmistress's chair and table, ceiling, bed linen, staff and children's skin and nostrils, children's chairs, drinking water and wash water. All specimens, except water, were collected using a sterile cotton swab stick previously moistened with saline (0.85% NaCl, ANALAR). Samples were labeled and transported to the laboratory within 2 hours for microbiological analysis.

Processing of samples

All specimens were identified with laboratory numbers. Except water, all other samples were inoculated on McConkey agar (Oxoid, England), *Staphylococcus* medium 110 (Oxoid, England); TCBS (Thiosulphate-Citrate Bile Salts Sucrose) medium and Nutrient agar for the isolation of coli aerogenes-like organisms (enterics), *Staphylococcus aureus*, *Vibrio cholerae* and for general purposes respectively. One hundred milliliters of the water samples was millipore filtered using a 0.45 µm pore size membrane filter (Millipore corporation, England) and plates were incubated at 37°C for 24-48 hours for a total coliform count and another set at 44 ± 0.5°C for 48 hours for a fecal coliform count (*E. coli*) in a thermostatically controlled water bath (Gallenkamp, England). *Vibrio* species were isolated by enrichment culture techniques using alkaline peptone water (pH 6) and incubated at 35°C for 6 hours. Subcultures were then made on TCBS medium at 37°C for 24 hours incubation. Similar enrichment culture techniques were used for isolation of *Salmonella* – *Shigella* species using Selenite-F broth before subcultures were made on S-S agar.

Identification of isolates

Since primary isolation yielded a mixed

population of organisms, pure cultures were made on fresh media by repeated subculture using streak plating techniques. Purified colonies were stored in slants at 4°C and thereafter characterized and identified using the standard taxonomic schemes of Cowan (1985) and Holt *et al* (1994).

Antibiogram of isolates

This was carried out using a pure culture of the enterics and *S. aureus* as previously described (Itah and Opara, 1994; Itah *et al*, 1998; Itah, 1999).

RESULTS

A total of 31 samples were collected from each of the four day care centers and the results were as follows.

Identified isolates

The isolates were identified as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* species, *Citrobacter* species, *Proteus vulgaris* and *Proteus mirabilis*. *Salmonella* species, such as *S. typhi* and *S. paratyphi*; *Shigella dysenteriae* and *Vibrio* species were not encountered in this investigation. There was a high incidence of enteric bacteria and *S. aureus* in all the centers with a large reservoir in formites. Day care centers I and II harbored more organisms than III and IV.

Distribution of microorganisms in the day care centers

It can be seen from Table 1 that no *Vibrio* species was encountered in Aunty Chimmy's Day Care and Nursery, Eket. However, profuse growth of *Staphylococcus aureus* was obtained from cultures of chairs, skin, nostrils, children's tables, the headmistress' chair; moderate growth was obtained from floors, bed linen, ceiling, while scanty growth was obtained from used water, windows, and walls. No *S. aureus* was isolated from door handles, fans or drinking water. Lactose fermenters were found profusely growing on cultures from door handles, children's tables and headmistress's table. Moderate growth was found from children's chairs and scanty growth from drinking water, fans and windows.

No lactose fermenters were found from used water, skin, floor, the headmistress' chair, walls and bed linen. There was profuse growth of non-

Table 1
Distribution of enteric bacteria and *Staphylococcus aureus* in Aunty Chimmy's Day Care and Nursery School, Eket.

Sources of specimens	No. of specimens	Nutrient agar	Growth pattern on			
			Mac Conkey agar		Mannitol salt agar (<i>S. aureus</i>)	TCBS (<i>Vibrio</i>)
			Lactose fermenter	Non-lactose fermenter		
Floors	2	++	-	-	++	-
Chairs	5	+	++	++	+++	-
Skin (girls)	2	+++	-	+	+++	-
Skin (boys)	2	+	-	+++	+++	-
Bed linen	2	++	-	-	++	-
Door handles	2	++	+++	+++	-	-
Fans	2	+	+	+	-	-
Children's tables	2	+++	+++	+++	+++	-
Walls	2	+	-	+++	+	-
Ceiling	1	++	-	-	++	-
Headmistress' table	1	+++	+++	-	-	-
Headmistress' chair	2	+++	-	-	+++	-
Windows	2	+	+	++	+	-
Drinking water	2	+++	+	+++	-	-
Used water	2	+++	-	+++	+	-
Total	31					

+++ = profuse growth; ++ = moderate growth; + = scanty growth; - = no growth

Table 2
Distribution of enteric bacteria and *Staphylococcus aureus* in Ideal Day Care and Nursery School, Eket.

Sources of specimens	No. of specimens	Nutrient agar	Growth pattern on			
			Mac Conkey agar		Mannitol salt agar (<i>S. aureus</i>)	TCBS (<i>Vibrio</i>)
			Lactose fermenter	Non-lactose fermenter		
Floors	2	+++	+	+	+++	-
Chairs	5	+	+	+	+++	-
Skin (girls)	2	+++	-	-	+++	-
Skin (boys)	2	++	-	+	+++	-
Bed linen	2	++	+	+	+++	-
Door handles	2	++	+++	+++	-	-
Fans	2	+	+	+	-	-
Children's tables	2	+++	+++	+++	+++	-
Walls	2	+	+	++	+	-
Ceiling	1	++	-	-	+	-
Headmistress' table	1	+	+	-	-	-
Headmistress' chair	2	+++	+	+	+++	-
Windows	2	+	+	+	+	-
Drinking water	2	+++	+	+++	-	-
Used water	2	+++	+	-	+++	-
Total	31					

+++ = profuse growth; ++ = moderate growth; + = scanty growth; - = no growth

lactose fermenters from cultures of boys' skin, door handles, children's tables, walls, drinking water and used water. Similar results have been presented for the various other day care centers for enteric bacteria and *S. aureus* (in Tables 2-7). The results show that *Escherichia coli* was the most frequently occurring enteric with a percentage incidence of 10.2-51.6% (Table 6). A summary of the incidence of enteric bacteria and *S. aureus* in the four Day Care Centers under investigation is presented in Table 7. The results show that *S. aureus* was the most frequently occurring organism (33.9%) followed by *E. coli* (19.0%), *Klebsiella* (14.4%), *Proteus vulgaris* (12.5%), *Citrobacter* (12.5%), and *Proteus mirabilis* (7.4%).

Antibiogram of isolates

Enteric bacteria exhibited a percentage sensitivity of 75 to 100 to second generation cephalosporins (ceftazidime and cefuronine). Others exhibited various sensitivity patterns to tetracycline, gentamicin, ampicillin, chloramphenicol

and cotrimoxazole (Table 8). All the *S. aureus* isolates from all the day care centers were resistant to benzyl-penicillin. Only strains from Adiaha Obong Day Care Center (DC IV) exhibited 100% sensitivity to ceftazidime and cefuronine antibiotics. Isolates from other day care centers exhibited multiple resistance and sensitivity patterns to other antibiotics, particularly tetracycline and streptomycin (Table 9).

DISCUSSION

The results obtained from this work have revealed the presence of enteric bacteria and *Staphylococcus aureus* in all the day care centers in varying proportions. The organisms encountered were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* species, *Citrobacter* species, *Proteus vulgaris* and *Proteus mirabilis*. The health risks associated with *Escherichia coli* and *Staphylococcus aureus* have already been well documented by earlier investigators (Anyiliffe *et al*, 1967; 1973;

Table 3
Distribution of enteric bacteria and *Staphylococcus aureus* in Trinity International Nursery School, Ikot Ekpene.

Sources of specimens	No. of specimens	Nutrient agar	Growth pattern on			
			Mac Conkey agar		Mannitol salt agar (<i>S. aureus</i>)	TCBS (<i>Vibrio</i>)
			Lactose fermenter	Non-lactose fermenter		
Floors	2	+++	+	+	+++	-
Chairs	5	++	-	-	+	-
Skin (girls)	2	++	-	-	+++	-
Skin (boys)	2	+	-	-	+	-
Bed linen	2	++	-	+	+	-
Door handles	2	+	+	+	-	-
Fans	2	++	-	+	-	-
Children's tables	2	+++	++	++	+++	-
Walls	2	++	+	+	+	-
Ceiling	1	++	-	+	-	-
Headmistress' table	1	+	-	+	+	-
Headmistress' chair	2	+	-	-	+	-
Windows	2	+	+	-	++	-
Drinking water	2	+	-	-	+	-
Used water	2	+	+	-	-	-
Total	31					

+++ = profuse growth; ++ = moderate growth; + = scanty growth; - = no growth

Table 4
Distribution of enteric bacteria and *Staphylococcus aureus* in Adiaha Obong Day Care and Nursery School, Uyo.

Sources of specimens	No. of specimens	Nutrient agar	Growth pattern on			
			Mac Conkey agar		Mannitol salt agar (<i>S. aureus</i>)	TCBS (<i>Vibrio</i>)
			Lactose fermenter	Non-lactose fermenter		
Floors	2	+	-	-	+	-
Chairs	5	+	-	+	-	-
Skin (girls)	2	+	-	-	+	-
Skin (boys)	2	+	+	-	+	-
Bed linen	2	++	+	+	-	-
Door handles	2	+	-	-	-	-
Fans	2	++	+	+	++	-
Children's tables	2	+	-	-	+	-
Wall	2	+	-	+	-	-
Ceiling	1	+	-	+	-	-
Headmistress' table	1	+	-	+	+	-
Headmistress' chair	2	+	-	+	-	-
Windows	2	+	-	-	-	-
Drinking water	2	-	-	-	-	-
Used water	2	+	+	-	-	-
Total	31					

++ = moderate growth; + = scanty growth; - = no growth

Table 5
Distribution of *Staphylococcus aureus* in four Day Care Centers in Akwa Ibom State.

Sources of specimens	No. of specimens collected	Total no. of specimens collected	Number of <i>Staphylococcus aureus</i> isolated			
			DCI	DCII	DCIII	DCIV
Floors	2	8	2	2	2	2
Chairs	5	20	5	5	5	0
Skin (girls)	2	8	2	2	2	2
Skin (boys)	2	8	2	2	2	2
Bed linen	2	8	2	2	2	2
Door handles	2	8	0	0	2	0
Fans	2	8	0	0	0	0
Children's tables	2	8	2	2	0	2
Walls	2	8	2	2	2	1
Windows	2	8	2	1	1	0
Ceiling	2	8	0	0	0	0
Headmistress' table	1	4	1	1	0	0
Headmistress' chair	1	4	1	1	1	1
Drinking water	2	8	0	0	1	0
Used water	2	8	2	0	0	0
Total	31	124	23	20	18	12

Table 6
Incidence of enteric bacteria isolated at four day care centers.

Sources of specimen	No. of samples treated in each center	No. of enteric bacteria isolated																							
		Vibrio			E. coli			Klebsiella			Citrobacter			P. vulgaris			P. mirabilis								
		AC	ID	TI	AO	AC	ID	TI	AO	AC	ID	TI	AO	AC	ID	TI	AO	AC	ID	TI	AO	AC	ID	TI	AO
Floor	2	0	0	0	2	1	0	1	0	2	1	2	1	2	0	1	1	1	0	1	1	0	1	0	0
Chairs	5	0	0	0	4	3	2	0	0	1	3	0	0	3	0	0	0	0	0	2	0	0	2	0	0
Skin (girl)	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin (boy)	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Bed linen	2	0	0	0	1	2	1	1	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0
Door handles	2	0	0	0	2	1	0	2	0	1	0	1	1	0	2	0	1	0	1	0	1	0	1	0	1
Fans	2	0	0	0	0	0	2	0	0	0	0	2	0	0	0	1	0	0	1	0	0	1	0	0	1
Children's tables	2	0	0	0	2	2	1	2	2	1	2	2	0	2	2	0	2	1	0	1	0	1	0	0	2
Wall	2	0	0	0	1	1	0	1	1	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0
Window	2	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0
Ceiling	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Headmistress' table	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0
Headmistress' chair	1	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	1	0	0	1	0	0	1	0	0
Drinking water	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Used water	2	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	0	1	0	0	2	0	0
Total	31	0	0	0	14	16	7	4	9	10	8	4	8	9	7	3	18	7	5	2	12	0	4	4	2
(%)	(0)	(0)	(0)	(0)	(45.2)	(51.6)	(22.6)	(10.2)	(29.3)	(32.2)	(25.8)	(10.2)	(25.8)	(29.8)	(22.6)	(9.8)	(69)	(22.6)	(7.0)	(30)	(12.9)	(0)	(0)	(12.9)	(7.0)

AC=Aunty chimney's Day Care and Nursery School, Eket.
 ID=Ideal Day Care and Nursery School, Eket.
 TI=Trinity International Nursery School, Ikot Ekpene.
 AO=Adiaha Obong Day Care Center, Uyo.

Table 7

Summary of incidence of enteric bacteria and *Staphylococcus aureus* isolated from four Day Care Centers in Akwa Ibom State.

Organisms	No. of specimens analysed	DCI	DCII	DCIII	DCIV	Total No. (%) of bacteria isolated among 124 samples
<i>Escherichia coli</i>	31	14 (18.18)	16 (20.7)	4 (11.4)	7 (16.2)	41 (19.0)
<i>Klebsiella</i> sp	31	9 (11.6)	10 (16.1)	4 (11.4)	8 (18.6)	31 (14.4)
<i>Citrobacter</i> sp	31	8 (10.3)	9 (14.5)	3 (8.5)	7 (16.2)	27 (12.5)
<i>Proteus vulgaris</i>	31	13 (16.8)	7 (11.2)	2 (5.7)	5 (11.6)	27 (12.5)
<i>Proteus mirabilis</i>	31	10 (12.9)	0 (0)	2 (5.7)	4 (9.3)	16 (7.4)
<i>Vibrio</i> sp	31	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>Staphylococcus aureus</i>	31	23 (29.8)	20 (32.2)	18 (15.4)	12 (27.9)	73 (33.9)
Total	124	77	62	35	43	215

Numbers in parenthesis represents percentage incidence.

Table 8

Antibiotic sensitivity pattern of enteric bacteria isolated from four Day Care Centers in Akwa Ibom State.

Antibiotics	No/Percentage sensitivity (%) among isolated strains							
	DCI		DCII		DCIII		DCIV	
	<i>E. coli</i> N=8	<i>Proteus</i> sp N=5	<i>E. coli</i> N=6	<i>Proteus</i> sp N=5	<i>E. coli</i> N=4	<i>Proteus</i> sp N=4	<i>E. coli</i> N=5	<i>Proteus</i> sp N=6
Tetracycline	0(0)	0	0	0	0	0	0	2(33.3%)
Ampicillin	5(62.5%)	2(40%)	4(66.6%)	4(80%)	3(75%)	4(100%)	5(100%)	4(66.6%)
Gentamicin	8(100%)	4(80%)	6(100%)	5(100%)	4(100%)	3(75%)	5(100%)	3(50.5%)
Cotrimoxazole	4(50%)	3(60%)	2(33.3%)	3(60%)	2(50%)	3(75%)	2(40%)	3(50.5%)
Chloramphenicol	3(33%)	2(40%)	3(50.5%)	3(60%)	1(25%)	2(50.5%)	1(20%)	2(33.3%)
Ceftazidine	8(100%)	5(100%)	6(100%)	5(100%)	4(100%)	3(75%)	4(75%)	5(100%)
Cefuronine	8(100%)	5(100%)	6(100%)	5(100%)	3(75%)	3(75%)	4(75%)	5(75%)

Table 9

Antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from four Day Care Centers in Akwa Ibom State.

Antibiotics		No/Percentage sensitivity (%) among isolated strains			
		DCI N = 17	DCII N = 11	DCIII N = 8	DCIV N = 6
Benzylpenicillin	1.5TV	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tetracycline	10mg	3 (17.6%)	3 (27.3%)	4 (50%)	2 (33.3%)
Streptomycin	10mg	4 (23.5%)	4 (36.4%)	5 (62.5%)	3 (50%)
Gentamicin	10mg	14 (82.4%)	8 (72.7%)	6 (75%)	5 (83.3%)
Erythromycin	10mg	12 (70.6%)	7 (63.6%)	8 (100%)	4 (66.7%)
Cotrimoxazole	25mg	11 (64.7%)	6 (54.5%)	7 (87.5%)	3 (50%)
Chloramphenicol	10mg	7 (41.2%)	5 (45.5%)	5 (62.5%)	4 (66.7%)
Ceftazidine	10mg	13 (76.5%)	8 (72.7%)	7 (87.5%)	6 (100%)
Cefuronine	10mg	12 (70.6%)	7 (63.6%)	7 (87.5%)	6 (100%)
Vancomycin	10mg	10 (58.8%)	6 (54.5%)	5 (62.5%)	4 (66.7%)

N=Number of strains tested.

Bergdoll, 1981; Chesney, 1981; Freeman, 1985; Anozie and Antai, 1987; Itah and Opara, 1997; Itah *et al*, 1998; Itah, 1991; 1999). Other enteric bacteria encountered in this work have been implicated in urinary tract infections (UTI) and are opportunistic pathogens of man (Cruikshank *et al*, 1975; Snyderman, 1989; Itah *et al*, 1998). Therefore, their presence in day care centers constitutes a health risk to staff and children in these centers. Anyliffe *et al* (1973) and Freeman (1985) reported that large numbers of *S. aureus* are disseminated from wounds and burns into the environment and that the organisms grow on moist skin and nostrils of 10-30% of healthy carriers. Chessbrough (1991) reported that most enteric bacteria, such as *E. coli*, *Klebsiella*, *Citrobacter* and *Proteus*, are ubiquitous and that these organisms can be shed from the body, clothing, beddings, nostrils and carried in the dust particles to other surfaces, hence their presence on the ceiling, fans, windows and door handles. These views have been shared by Anyliffe *et al* (1973).

The fact that *S. aureus* is a normal flora of the skin and the body probably explains its high percentage of contamination (33.9%) as they are easily discharged while sneezing, talking and through moist skin (during sweating) and clothing. Enteropathogenic *Escherichia coli* is a well known agent of infantile and traveller's diarrhea, therefore its presence in the centers poses a health risk, as it can contaminate fruits, food and feeding bottles used by children. There is a need for continuous surveillance of these centers to avert an outbreak of enteric pathogens and *S. aureus*. The enforcement of an effective public health enlightenment program is advocated in order to attract sufficient attention of the proprietors of these establishments to the role of fomites as reservoirs of enteric microorganisms.

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