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Bacteriological Study of Coagulase Positive Staphylococci from Urine in Some Akoko Communities, Ondo State, Nigeria

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Abstract: *Staphylococci* which can be normal skin flora as well as aetiologic agent was examined for their clinical implications from urine sources during the study. The occurrence of staphylococcal species in urine sources of some people in Akoko communities between age group 5 to 55 years was determined. 50 sample sources were screened for this purpose. The total viable bacterial count of urine sample sources range from 0, 1 x 10^3 cfu/ml and 4 x 10^3 cfu/ml from Ikun-Akoko, Supare community, Iwaro-Akoko as well as Akungba-Akoko respectively to high microbial load of 50 x 10^6 cfu/ml from Supare-Akoko. The bacterial isolates were identified using standard microbiological techniques including biochemical characteristics in which 23 (46%) were coagulase positive and 25 (50%) were coagulase negative and 2 (4%) show no growth on Mannitol Salt Agar. The 48 isolates obtained for this study were all Gram positive cocci (Bacteria). The Staphylococcal species obtained were grouped as C.P.S a 1:- Coagulase positive *Staphylococcus aureus* group 1, C.P.S.a 2:- Coagulase positive *Staphylococcus aureus* group 2, C.NS 1; Coagulase negative Staphylococcus group 1, C.N.S 2: Coagulase negative Staphylococcus group 2 and C.N.S 3: Coagulase negative Staphylococcus group 3. Some of the isolates tested show multiple antibiotic resistance which is significant clinically in case of treatment of infections in immunocompromised persons. The physico-chemical properties of urine samples collected from the Akoko communities studied was determined using Combi 9 strip/kit. This study will help to generate a database for health management purposes with the aim of controlling *Staphylococcus* infections.

Keywords: Akoko communities; Coagulase positive staphylococci; Infections; Urine; Nigeria.

1. Introduction

Worldwide, about 150 million people are diagnosed with UTI each year costing the global economy in excess of 6 billion US dollars. It is a serious ailment in human due to the frequency, recurrence and difficulty in eradication [1]. The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria been massaged up the urethra and into the bladder during pregnancy and or child birth [2].

Nowadays, drug resistance is a huge growing problem in treating infectious diseases like malaria, tuberculosis, diarrheal disease and urinary tract infections (UTIs) etc. Typical example of such resistant strains are, methicillin resistant *Staphylococcus aureus* (MRSA), multidrug resistant *Pseudomonas aeruginosa* and *Serratia marcescens*, vancomycin resistant enterococci (VRE) and extended spectrum beta lactamase (ESBL) resistant Enterococci [3]. Drug resistance of pathogens is a serious medical problem, because of very fast arise and spread of mutant strains that are resistant to medical treatment. The highest incidence of urinary tract infection occurs in the child bearing age and this has been linked directly to sexual activity and aging [4]. The urinary tract is comprised of the kidneys, bladder, ureters and urethra. The Urinary Tract Infections (UTI) are caused by pathogenic organisms in any of the structures. Many other infections such as arthritis (urethral infection), cystitis (bladder infection), ureters infection and pyelonephritis (kidney infection) and other structures that eventually connect to or share close anatomical proximity to the urinary tract (for example, prostate, vagina and epididymis) are sometimes included in the discussion of UTIs. Urinary tract infection caused by bacteria affects any part of the urinary tract [5].

Staphylococcus aureus is one of the most important opportunistic pathogen among Staphylococci belonging to Micrococaceae family causing significant infections under appropriate conditions [6]. Although *Staphylococcus aureus* is an important pathogen, many healthy people may carry it as a part of the normal microflora associated with the nose, throat, perineum or skin [6].

The species most commonly implicated as the etiological agent in infections of humans and animals is the coagulase-positive *Staphylococcus aureus*. The coagulase-negative staphylococci (CNS) are, as a group the most frequently encountered bacteria in medical microbiology laboratories and have been considered to be saprophytic and rarely pathogenic [7]. More recently however, CNS have emerged as significant pathogens, particularly in

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infections associated with medical devices and in immunocompromised patients. In early studies, *Staphylococcus epidermidis, Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* were associated with human infections [7].

More recently numerous reports have implicated a much wider range of species as etiological agents associated with confirmed infectious processes. *Staphylococcus epidermidis* is extremely well documented as a pathogen in cases of bacteraemia, prosthetic valve endocarditis, urinary tract, cerebrospinal fluid, peritoneal fluid and prosthetic joint infections. *Staphylococcus haemolyticus* is the second most commonly encountered CNS associated with human infection, being implicated in cases of native – valve endocarditis, septicaemia, wound, bone and joint infections and urinary tract infections. *Staphylococcus saprophyticus* is also well recognised as an opportunistic pathogen in urinary tract infections, particularly in young sexually active females. *Staphylococcus lugdunensis* is also implicated in native- valve infections as well as arthritis, catheter, prosthetic joint and urinary tract infections. The other species of CNS implicated in human infections have been associated with endocarditis, septicaemia, pneumonia, wound and joint infections and osteomyelitis [8]. The objectives of this study are to determine the prevalence of coagulase positive *Staphylococcus aureus* among people in Akoko South West Local Government Area from urine samples and the determination of the antibiotic susceptibility profile of the isolates. Similarly, investigation of the effect coagulase positive *Staphylococcus aureus* on the physico-chemical properties of the urine using combi-9 was intensified.

2. Materials and Method

2.1. Study Site

The study area covers four town in Akoko south west local government, Ondo state which include; Akungba, Supare, Iwaro-Oka and Ikun-Akoko. The majority of the residents of Akoko south west area are famers, civil servants, artisan and students. The study was carried out in Adekunle Ajasin University Health Centre, Akungba-Akoko, Ajagbokun High School, Ikun-Akoko and hospitals. Randomized samples were collected from the university students, secondary school students, children, artisan, and civil servants. Fifty samples were collected in all within Akoko south west covering four towns in all and the materials used for the study were adequately sterilized.

2.2. Samples Collection

A total of fifty mid-stream urine Samples were collected from the subject into sterile universal bottle which, having been instructed on how to collect the urine samples which consist of 30 female (%) and 20 (%) male. The samples were then transferred within 30 minutes of collection to laboratory for analysis.

2.3. Media and Reagent Used

The culture medium routinely used for the study include; Mannitol Salt agar, blood agar, nutrient agar and Muller Hinton agar. Reagents used include; Hydrogen peroxide, Gram staining reagents (including safranine, 70% alcohol, crystal violet, and Lugols iodine).

2.4. Urine Culturing

Using 10^{-5} dilution factor, 1ml of the diluents was pipette and gently transferred into a sterile Petri dish aseptically. The sterilized Mannitol salt medium was then poured gently into the Petri dish containing the diluents and was gently rocked in both clockwise and anticlockwise manner. The inoculated plates were then incubated at 37^{o}C for 24 hours. The same procedure was used for all other samples.

2.5. Bacterial Burden of Urine

Ten-fold serial dilution were made by transferring 1.0ml of the urine samples into 9.0ml of sterile distilled water and a ratio of $1:10^{-3}$ and $1:10^{-6}$ aliquot of each dilute samples (0.1ml) were spread on surface or dispensed into molten manitol salt agar in the petri dishes and gently mixed, uniformly distributed. The plates were incubating at 37^{o}C for 24 hours. Bacteria colonies on the plates were enumerated to the determined microbial load (bacteriuria).

2.6. Isolation and identification of bacteria from sample

Standard microbiological techniques were used to identify the bacterial isolates. A loopful of each urine sample was streaked in Manito salt agar(selective media) to isolate only Staphylococci which were transferred to Blood agar to differentiate beta from alpha haemolysis and were further confirms by biochemical tests.

2.7. Antimicrobial Susceptibility Testing

The bacteria were tested using the following antibiotics to determine their susceptibility pattern. Such as: Ceftzidime 30µg, Cefuroxine 30µg, Gentamicin 10µg, Ciprofloxacin 5µg, Ofloxacin 5µg, Amoxycillin/clavulanate 30µg, Nitrofurantoin 300µg, e.t.c were determined by using agar disc diffusion method as described by Bauer, *et al.* [9].

The inoculated plates containing the antibiotics were incubated 37^{o}C for 24 hours, after which the diameter of zone of inhibition around each antibiotic disc were then measured to the nearest millimetre and interpreted according to the current CLSI standard [10].

3. Results

The study shows the occurrence of staphylococcal species in urine sources of some people in Akoko communities between age group 5-55years. Sum of 50 samples were examined for this purpose. Twenty three (46%) were Coagulase positive *Staphylococcus aureus,* 25 (50%) were Coagulase negative Staphylococci and 2 (4%) show no growth on the culture media used (MSA) (Table 1). The total viable bacterial count of urine sample sources range from 0, 1 x 10^3 cfu/ml and 4 x 10^3 cfu/ml from Ikun-Akoko, Supare community, Iwaro-Akoko as well as Akungba-Akoko respectively to high microbial load of 50 x 10^6 cfu/ml from Supare-Akoko (Table 2 to 5). In Table 6 to 9, the physico-chemical properties of urine samples collected from the Akoko communities studied was determined using Combi 9 strip/kit.

Table 10 to 13 shows the morphological characteristics and some biochemical test for all the isolates. In Table 14, the pathogenic occurrence pattern of the isolates was determined, in which 23 (46%) were coagulase positive and 25 (50%) were coagulase negative.

A total number of 48 isolates were obtained and they were all Gram positive Cocci (Bacteria). In Table 15, the antibiotic susceptibility tests of Staphylococcal species obtained was determined and were grouped as follow: C.P.S a 1:- Coagulase positive *Staphylococcus aureus* group 1, C.P.S.a 2:- Coagulase positive *Staphylococcus aureus* group 2, C.NS 1; Coagulase negative Staphylococcus group 1, C.N.S 2: Coagulase negative Staphylococcus group 2, C.N.S 3: Coagulase negative Staphylococcus group 3. Figure 1 shows the percentages of coagulase positive staphylococci isolated from each town while figure 2 shows the percentages of the grouped isolates.

Table-1. Prevalence of coagulase positive *Staphylococcus aureus* within the studied age range

Age group	Total number of screened	No of positive and percentage (%)
5 – 15	10	3 (6%)
16 -25	20	8 (16%)
26 – 35	10	6 (12%)
36 – 45	5	3 (6%)
46 – 55	5	3 (6%)
Total	50	23 (46%)

Table-2. Total viable bacteria count of the urine sample in Akungba-Akoko.

S/N	Sex	10^{-3} Cfu/ml	10^{-6} Cfu/ml
AK1	F	25	10
AK2	F	40	20
AK3	M	31	20
AK4	F	47	23
AK5	M	16	10
AK6	M	24	10
AK7	M	19	09
AK8	F	45	25
AK9	F	32	25
AK10	F	46	26
AK11	F	04	01
AK12	F	04	_
AK13	F	07	03
AK14	M	19	11
AK15	M	35	25
AK16	F	08	02
AK17	M	39	21
AK18	F	10	04
AK19	M	14	11
AK20	M	18	10

Legend: AK= Code for samples collected in Akungba-Akoko , F=Female and M=Male.

Table-3. Total viable bacteria count of the urine sample in Iwaro-Oka-Akoko.

S/N	Sex	10^{-3} Cfu/ml	10^{-6} Cfu/ml
IW1	M	23	09
IW2	M	01	_
IW3	F	04	_
IW4	M	06	02
IW5	M	17	08
IW6	F	11	09
IW7	M	12	10
IW8	F	14	09
IW9	M	04	_
IW10	M	19	11

Legend: IW= Code for samples collected in Iwaro-Oka-Akoko , F=Female and M=Male.

Table-4. Total viable bacteria count of the urine sample in Ikun-Akoko.

S/N	Sex	10^{-3} Cfu/ml	10^{-6} Cfu/ml
IK1	F	15	05
IK2	F	48	17
IK3	F	_	_
IK4	M	17	13
IK5	F	16	09
IK6	F	31	19
IK7	M	19	16
IK8	F	08	02
IK9	F	05	_
IK10	M	15	11

Legend: IK= Code for samples collected in Ikun-Akoko, F=Female and M=Male.

Table-5. Total viable bacteria count of the urine sample in Supare- Akoko.

S/N	Sex	10^{3} Cfu/ml	10^{6} Cfu/ml
SU1	F	TNC	50
SU2	F	05	01
SU3	M	15	05
SU4	F	10	05
SU5	F	16	09
SU6	F	08	02
SU7	F	_	_
SU8	F	15	06
SU9	F	21	14
SU10	F	05	_

Legend: SU= Code for samples collected in Supare-Akoko , F=Female and M=Male.

Table-6. Physico-chemical properties of urine samples collected in Akungba-Akoko.

S/N	Colour	Appearance	Blood	Urobilinogen	Bilirubin	Protein	Nitrite	Ketone	Glucose	Ascorbic acid	pH
AK1	Y	Turbid	_	_	_	_	_	_	_	_	7.0
AK2	S	Clear	+	+	_	_	_	_	_	_	8.0
AK3	A	Turbid	_	_	_	+	_	_	_	_	7.0
AK4	Y	Clear	_	_	_	_	_	_	_	+	5.0
AK5	A	Clear	_	_	_	_	_	_	_	_	7.0
AK6	Y	Clear	_	_	_	_	_	_	_	_	7.0
AK7	A	Cloudy	_	_	_	+	_	_	_	_	8.0
AK8	A	Cloudy	_	_	_	_	_	_	_	_	7.0
AK9	A	Clear	_	_	_	_	_	_	_	_	5.0
AK10	Y	Clear	_	_	_	_	_	_	_	_	5.0
AK11	Y	Clear	_	_	_	_	_	_	_	_	6.0
AK12	Y	Cloudy	_	_	_	_	_	_	_	_	7.0

AK13	A	Clear	_	_	_	_	+	_	_	_	7.0
AK14	S	Clear	_	_	_	_	_	_	_	_	5.0
AK15	Y	Clear	_	_	_	_	_	_	_	_	6.0
AK16	Y	Clear	_	_	_	_	_	_	_	_	7.0
AK17	Y	Clear	_	_	_	_	_	_	_	_	6.0
AK18	A	Cloudy	_	_	_	_	_	_	_	_	6.0
AK19	Y	Clear	_	_	_	_	_	_	_	+	5.0
AK20	Y	Clear	_	_	_	_	_	_	_	_	7.0

Legend: AK= Code for samples collected in Akungba-Akoko, Y=Yellow, A= Amber, - (Negative) and + (Positive).

Table-7. Physico-chemical properties of urine samples collected in Iwaro-Akoko.

S/N	Colour	Appearance	Blood	Urobilinogen	Bilirubin	Protein	Nitrite	Ascorbic acid	Ketone	Glucose	pH
IW1	Y	Cloudy	_	_	_	_	_	_	_	_	7.0
IW2	Y	Clear	_	_	_	_	_	_	_	_	6.0
IW3	Y	Clear	_	_	_	_	_	_	_	_	6.0
IW4	A	Cloudy	_	_	_	+	+	_	_	_	8.0
IW5	Y	Cear	_	_	_	_	_	_	_	_	6.0
IW6	Y	Clear	_	_	_	_	_	_	_	_	7.0
IW7	Y	Clear	_	_	_	_	_	_	_	_	7.0
IW8	Y	Clear	_	_	_	_	+	_	_	_	7.0
IW9	Y	Clear	_	_	_	_	_	_	_	_	7.0
IW10	Y	Clear	_	_	_	_	_	_	_	_	7.0

Legend: IW= Code for samples collected in Iwaro-Oka-Akoko, Y=Yellow, A= Amber, - (Negative) and + (Positive).

The table shows the physico-chemical properties of each urine sample which were done using Combi-9 test strip. Appearance and colour were done by observing with naked eyes.

Table-8. Physico-chemical properties of urine samples collected in Ikun-Akoko.

S/N	Colour	Appearance	Blood	Urobilinogen	Bilirubin	Protein	Nitrite	Ascorbic acid	Ketone	Glucose	pH
IK1	Y	Cloudy	_	_	_	+	_	_	_	_	7.0
IK2	Y	Clear	_	_	_	___	_	+	_	_	6.0
IK3	Y	Clear	_	_	_	_	_		_	_	5.0
IK4	Y	Clear	_	_	_	_	_	_	_	_	8.0
IK5	Y	Clear	_	_	_	_	_	_		_	7.0
IK6	Y	Clear	_	_	_	+	_	_	_	_	7.0
IK7	A	Clear	_	_	_	_	_	_	_	_	8.0
IK8	Y	Clear	_	_	_	_	_	_	_	_	7.0
IK9	Y	Clear	_	_	_	_	_	_	_	_	7.0
IK10	Y	Clear	_	_	_	_	_	+	_	_	6.0

Legend: IK= Code for samples collected in Ikun-Akoko, Y=Yellow, A= Amber, - (Negative) and + (Positive).

Table-9. Physico-chemical properties of urine samples collected from Supare-Akoko.

S/N	Appearance	Colour	Blood	Urobilinogen	Bilirubin	Protein	Nitrite	Ascorbic acid	Ketone	Glucose	p H
SU1	Clear	Y	_	_	_	_	_	_	_	_	7.0
SU2	Clear	Y	_	_	_	_	_	_	_	_	7.0
SU3	Clear	Y	_	_	_	_	_	_	_	_	6.0
SU4	Clear	A	_	_	_	_	_	_	_	_	7.0
SU5	Cloudy	Y	_	_	_	_	_	_	_	_	8.0
SU6	Clear	Y	_	_	_	_	_	+	_	_	5.0
SU7	Clear	A	_	_	_	_	_	_	_	_	5.0
SU8	Cloudy	S	_	_	_	_	_	_	_	_	7.0
SU9	Clear	Y	_	_	_	_	_	_	_	_	7.0
SU10	Clear	Y	_	_	_	_	+	_	_	_	6.0

Legend: SU= Code for samples collected in Supare-Akoko, Y=Yellow, A= Amber, - (Negative) and + (Positive).

Table-10. Morphological Characteristics of the isolated organisms from urine samples collected in Akungba-Akoko.

S/N	Cultural morphology on MSA	Blood heamolysis	Gram staining	Shapes and arragement	COA	CAT	OX	SUC	FRU	LAC	GLU	XYL	GRAM	SUSPECTED ORGANISM
AK1 (C)	White-yellow, smooth circular colony	Gamma	+	Cocci in cluster	_	+	+	ND	_	_	AG	AG	+	Coagulase negative *Staphylococcus*
AK2 (A)	Yellowish, mucoid irregular eadge colony	Beta	+	Cocci in cluster	+	+	+	_	AG	AG	AG	ND	+	*Staphylococcus aureus*
AK3 (A)	Golden yellow, mucoid circular colonies	Beta	+	Cocci in cluster	+	+	+	A	AG	AG	_	_	+	*Staphylococcus aureus*
AK4 (D)	Whitish , non-mucoid colonies	Gamma	+	Cocci in cluster	_	+	_	AG	AG	A	AG	_	+	Coagulase negative *Staphylococcus*
AK5 (D)	Whitish non-mucoid smooth colonies	Gamma	+	Cocci in cluster	_	+	_	_	AG	AG	ND	_	+	Coagulase negative *Staphylococcus*
AK6 (E)	Whitish yellow, non-mucoid circular colonies	Alpha	+	Cocci in cluster	_	+	_	A	_	AG	_	_	+	Coagulase negative *Staphylococcus*
AK7 (B)	Yellowish, non-mucoid circular colonies	Beta	+	Cocci in cluster	+	+	_	AG	A	AG	ND	_	+	*Staphylococcus aureus*
AK8 (B)	Whitish-yellow,non-mucoid	Beta	+	Cocci in cluster	+	+	_	AG	_	_	AG	_	+	*Staphylococcus aureus*

	circular with irregular eadge													
AK9 (C)	Yellowish , mucoid circular colonies	Gamma	+	Cocci in cluster	_	+	+	¬ _	A	A G	A G	A	+	Coagulase negative *Staphylococcus*
AK10 (C)	Whitish-yellow, big non-mucoid smooth colonies	Gamma	+	Cocci in cluster	_	+	+	¬ _	_	_	A G	A G	+	Coagulase negative *Staphylococcus*
AK11 (E)	Yellowish , raised circular, non-mucoid colonies	Alpha	+	Cocci in cluster	_	+	_	N D	_	_	_	A G	+	Coagulase negative *Staphylococcus*
AK12 (A)	Yellowish , circular mucoid colonies	Beta	+	Cocci in cluster	+	+	+	A G	A G	A	A	N D	+	*Staphylococcus aureus*
AK13 (B)	Whitish-yellow, non-mucoid circular colonies	Gamma	+	Cocci in cluster	+	+	_	A G	A G	A	_	_	+	*Staphylococcus aureus*
AK14 (ND)	White, raised irregular colonies	ND	+	Cocci in cluster	_	+	+	_	_	_	A	N D	+	Coagulase negative *Staphylococcus*
AK15 (C)	Whitish yellow, non-mucoid colonies	Gamma	+	Cocci in cluster	_	+	+	A G	A G	A G	_	A	+	Coagulase negative *Staphylococcus*
AK16 (E)	Smooth , raised white, circular colonies	Alpha	+	Cocci in cluster	_	+	_	N D	A	_	A	A G	+	Coagulase negative *Staphylococcus*
AK17 (A)	Yellow, raised mucoid colonies	Beta	+	Cocci in cluster	+	+	+	_	A G	A	A G	_	+	*Staphylococcus aureus*
AK18 (B)	Whitish-yellow, raised non-mucoid circular colonies	Beta	+	Cocci in cluster	+	+	_	A	A	A G	_	A G	+	*Staphylococcus aureus*
AK19 (ND)	Whitish yellow, non-mucoid circular colonies	ND	+	Cocci in cluster	_	+	_	A	_	A G	_	N D	+	Coagulase negative *Staphylococcus*
AK20 (C)	whitish-yellow mucoid	Gamma	+	Cocci in cluster	_	+	+	_	A G	A	_	A	+	Coagulase negative *Staphylococcus*

	smooth colonies													

Legend: (A) – C.P.S.A 1, (B)- C.P.S.A 2, (C)- C.N.S 1, (D)- C.N.S 2, (E)- C.N.S 3

Legend: OX- oxidase ,COA- coagulase, CAT- catalase, FRU- fructose, LAC- lactose, GLU- glucose, SUC-sucrose, XYL- xylose, +: posxitive, -: negative, A- acid, AG- acid and gas.

(A) – C.P.S.A 1, (B)- C.P.S.A 2, (C)- C.N.S 1, (D)- C.N.S 2, (E)- C.N.S 3

Table-11. Morphological Characteristics of the isolated organisms from urine samples collected from Iwaro-Oka-Akoko.

S/N	Cultural morphology on MSA	Blood heamolysis	Gram staining	Shape and arrangement	COA	CAT	OX	SUC	FRU	XYL	GLU	LAC	GRAM	SUSPECTED ORGANISM
IW1 (A)	Yellow raised mucoid circular colonies	Beta	+	Cocci in cluster	+	+	+	ND	_	ND	AG	A	+	*Staphylococcus aureus*
IW2 (C)	Whitish smooth circular colonies	Gamma	+	Cocci in cluster	_	+	+	_	A	AG	_	AG	+	Coagulase negative *Staphylococcus*
IW3 (B)	Whitish yellow, non-mucoid circular colonies with irregular eadge	Beta	+	Cocci in cluster	+	+	_	AG	_	AG	_	_	+	*Staphylococcus aureus*
IW4 (E)	Whitish yellow non-mucoid colonies	Alpha	+	Cocci in cluster	_	+	+	AG	AG	_	AG	A	+	Coagulase negative *Staphylococcus*
IW5 (A)	Yellow , big circular mucoid colonies	Beta	+	Cocci in cluster	+	+	_	ND	_	ND	AG	A	+	*Staphylococcus aureus*
IW6 (C)	Whitish non –mucoid smooth colonies	Gamma	+	Cocci in cluster	_	+	+	A	AG	A	AG	_	+	Coagulase negative *Staphylococcus*
IW7 (C)	Whitish-yellow	Gamma	+	Cocci in	_	+	+	_	AG	_	A	AG	+	Coagulase negative

	raised mucoid small circular colonies			cluster										*Staphylococcus*
IW8 (A)	Yellow mucoid , raised circular colonies	Beta	+	Cocci in cluster	+	+	+	A G	A G	_	_	A G	+	*Staphylococcus aureus*
IW9 (B)	Whitish yellow non mucoid smooth colonies	Beta	+	Cocci in cluster	+	+	_	A G	A G	A	A G	_	+	*Staphylococcus aureus*
IW10 (ND)	Whitish smooth circular colonies	ND	+	Cocci in cluster	_	+	_	A G	A G	ND	A G	A	+	Coagulase negative *Staphylococcus*

Legend: (A) – C.P.S.A 1, (B)- C.P.S.A 2, (C)- C.N.S 1, (D)- C.N.S 2, (E)- C.N.S 3

Table-12. Morphological Characteristics of the isolated organisms from urine samples collected in Ikun-Akoko.

S/N	**Cultural morphology on MSA**	**Blood heamolysis**	**Gram staining**	**Shapes and arrangement**	**COA**	**CAT**	**OX**	**SUC**	**FRU**	**XYL**	**GLUC**	**LAC**	**GRAM**	**SUSPECTED ORGANISM**
IK1 (A)	Yellow raised mucoid circular colonies	Beta	+	Cocci in cluster	+	+	+	A G	ND	ND	A G	A G	+	*Staphylococcus aureus*
IK2 (ND)	Whitish smooth small circular colonies	ND	+	Cocci in cluster	_	+	_	A G	A G	_	A G	A	+	Coagulase negative *Staphylococcus*
IK4 (A)	Yellowish mucoid circular colonies	Beta	+	Cocci in cluster	+	+	+	A G	ND	A	A G	A G	+	*Staphylococcus aureus*
IK5 (B)	Whitish-yellow non-mucoid circular colonies	Alpha	+	Cocci in cluster	+	+	_	A	_	ND	_	A G	+	*Staphylococcus aureus*
IK6 (A)	Yellowish mucoid circular colonies	Beta	+	Cocci in cluster	+	+	+	_	_	_	A	A G	+	*Staphylococcus aureus*
IK7	Whitish	Gamma	+	Cocci in	_	+	+	A	A	A	A	A	+	Coagulase

(C)	smooth, mucoid circular colonies			cluster								G		negative *Staphylococcus*
IK8 (B)	Yellow non mucoid circular colonies	Alpha	+	Cocci in cluster	+	+	_	A G	A	A	A G	A	+	*Staphylococcus aureus*
IK9 (C)	Whitish yellow non mucoid colonies	Gamma	+	Cocci in cluster	¬ _	+	+	N D	A G	_	A G	_	+	Coagulase negative *Staphylococcus*
IK10 (A)	Yellowish raised mucoid circular colonies	Bata	+	Cocci in cluster	+	+	+	_	A G	A G	A G	A	+	*Staphylococcus aureus*

Table-13. Morphological Characteristics of the isolated organisms from urine samples collected from Supare-Akoko.

S/N	**Cultural morphology on MSA**	**Blood heamolysis**	**Gram staining**	**Shapes and arrangement**	**COA**	**CAT**	**OX**	**GLU**	**FRU**	**SUC**	**LAC**	**XYL**	**GRAM**	**SUSPECTED ORGANISIM**
SU1 (A)	Yellowish mucoid circular colonies	Beta	+	Cocci in cluster	+	+	+	A G	A	A G	_	N D	+	*Staphylococcus aureus*
SU2 (C)	Yellowish non mucoid circular colonies	Gamma	+	Cocci in cluster	_	+	+	A G	_	A G	_	_	+	Coagulase positive *Staphylococcus*
SU3 (B)	Whitish yellow non mucoid smooth colonies	Beta	+	Cocci in cluster	+	+	_	_	A	A G	A	N D	+	*Staphylococcus aureus*
SU4 (E)	Whitish non mucoid circular colonies	Alpha	+	Cocci in cluster	_	+	+	N D	A G	A	A	A	+	Coagulase positive *Staphylococcus*
SU5 (A)	Whitish yellow mucoid big circular colonies	Beta	+	Cocci in cluster	+	+	+	A G	A G	A G	A G	_	+	*Staphylococcus aureus*
SU6 (D)	Whitish yellow non mucoid circular colonies	Gamma	+	Cocci in cluster	_	+	_	A G	N D	A	_	A G	+	Coagulase positive *Staphylococcus*
SU8 (E)	Yellowish non mucoid circular colonies	Alpha	+	Cocci in cluster	_	+	+	N D	A	N D	A	A	+	Coagulase positive *Staphylococcus*
SU9 (E)	Whitish small smooth circular colonies	Alpha	+	Cocci in cluster	_	+	_	A	A	A G	A G	A G	+	Coagulase positive *Staphylococcus*

SU10 (A)	Yellowish raised mucoid big circular colonies	Beta	+	Cocci in cluster	+	+	+	A G	A G	A G	A	A	+	*Staphylococcus aureus*

Legend: (A) – C.P.S.A 1, (B)- C.P.S.A 2, (C)- C.N.S 1, (D)- C.N.S 2, (E)- C.N.S 3
OX- oxidase ,COA- coagulase, CAT- catalase, FRU- fructose, LAC- lactose, GLU- glucose, SUC-sucrose, XYL- xylose, +: posxitive, -: negative, ND- not determined, A- acid, AG- acid and gas. (A) – C.P.S.A 1, (B)- C.P.S.A 2, (C)- C.N.S 1, (D)- C.N.S 2, (E)- C.N.S 3

Table-14. Groups of Staphylococcus obtained and their characteristics

Bacteria strains	Isolates	Percentage % and number	Characteristics
C.P.S.a 1	*Staphylococcus aureus*	14 (28%)	Beta hemolysis, oxidase positive, yellow mucoid colonies and show resistance to nitrofurantoin.
C.P.S.a 2	*Staphylococcus aureus*	09 (18%)	Some are beta, alpha, oxidase negative, mucoid and mostly non- mucoid, whitish-yellow and show resistance to Cloxacillin.
C.N.S 1	*Staphylococcus epidermidis*	11 (22%)	Gamma heamolysis, whitish-yellow, mainly small colonies, oxidase positive. They are susceptible to all the antibiotic except cefixime but highly susceptible to novobiocin
C.N.S 2	*Staphylococcus epidermidis*	03(6%)	Gamma heamolysis, oxidase negative, whitish to yellowish colonies and susceptible to all the antibiotics used.
C.N.S 3	*Staphylococcus saprophyticus*	07 (16%)	Alpha or beta heamolysis, oxidase positive, raised non mucoid colonies, resistance to gentamicin and novobiocin

Legend: C.P.S a 1:- Coagulase positive *Staphylococcus aureus* group 1, C.P.S.a 2:- Coagulase positive *Staphylococcus aureus* group 2, C.NS 1; Coagulase negative Staphylococcus group 1, C.N.S 2: Coagulase negative Staphylococcus group 2, C.N.S 3: Coagulase negative Staphylococcus group 3.
The isolates above were grouped based on reaction to antibiotics, cultural characteristics, oxidase coagulase and blood haemolysis.

Table-15. Antibiotic susceptibility pattern of the isolates

ISOLATES	NOV	ERY	NIT	OFL	CXC	GEN	AUG	CRX	CAZ	CTR	CXM
C.P.S.a1	15(s)	15(s)	R	18(s)	10 (i)	16 (s)	22 (s)	15 (s)	11 (i)	18 (s)	15 (s)
C.P.S.a2	14(i)	11(i)	10(i)	20(s)	R	11 (i)	18 (s)	12 (i)	14 (i)	15 (s)	11 (i)
C.N.S 1	20(s)	12(i)	21(s)	15(s)	15(s)	11 (i)	24 (s)	17 (s)	18 (s)	10 (i)	R
C.N.S2	15(s)	16(s)	22(s)	20(s)	18(s)	15 (s)	17 (s)	20 (s)	15 (s)	20 (s)	14 (i)
C.N.S.3	R	18(s)	17(s)	17(s)	11(i)	R	15 (s)	18 (s)	11 (i)	18 (s)	12 (i)

Legend: S: Sensitive, R: Resistance, I: Intermediate, ERY: Erythromycin, NIT: Nitrofurantoin, OFL: Ofloxacin, CXC: Cloxacilin, GEN: Gentamicin. AUG: Augumentin, CRX: Cefuroxime, CAZ: Ceftazidime, CTR: Ceftriazone, CXM: Cefixime, NOV: Nocobiocin.
The result from the table shows some of the isolates that are highly sensitive to some antibiotic with >15, some as intermediate <14 and some resistance i.e less than 10 (<10).

Figure-1. Percentage of coagulase positive staphylococcus aureus isolated from selected town

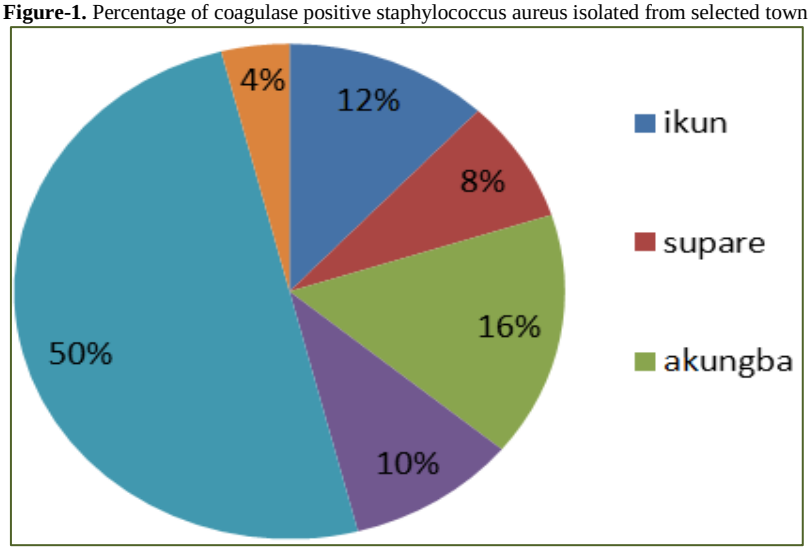
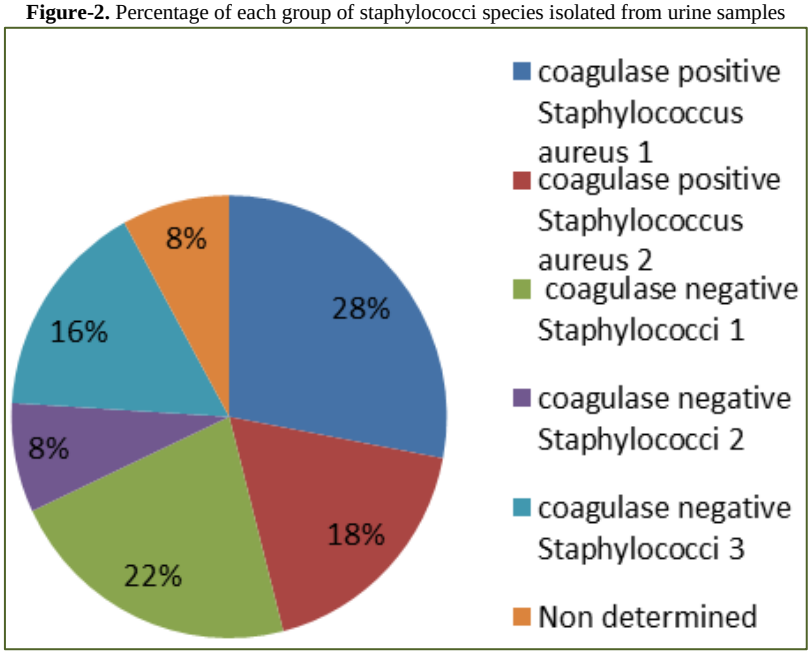


Figure-2. Percentage of each group of staphylococci species isolated from urine samples



4. Discussion

This study shows the epidemiological distribution of *Staphylococcus aureus* among people in Akoko South West Local government, Nigeria. In Table 1, the prevalence of significant Staphylococcus bacteriuria among people with age range 5-55 was determined. Highest number of *Staphylococcus aureus* was obtained from people of age range 16-23 years which was 8 out of 20 urine sample screened, 6 were obtained from age range 26-35 out of total urine samples of 10 screened, while in age range 5-15, 36-45and 46-55 were 3 respectively out of 10 urine samples screened for each group. This correlates to the study of Kunin [11].

The result from Table 2 to Table 5 shows that the bacterial population on mannitol salt agar varies for all the samples screened based on related physiological factors as exemplified under the pshysiological analysis. This collaborate with the work of Muder, *et al.* [12], who showed the bacteria burden of *Staphylococcus* in the isolation of *Staphylococcus aureus* from urine.

The result from Table 6-9 shows the physico-chemical properties of each urine samples collected using Combi-9 urine test strip. From the Table, coagulase positive *S. aureus* was isolated mostly from yellow colour, clear or cloudy in appearance, also nitrite and protein are mostly positive for the samples with pH range 6.0- 7.0 and are rarely above pH 7.0 which corroborates with the study of Khurana [13].

The result from Table 10 -13 shows the morphological and biochemical characteristics of the isolates which include Gram staining, shapes and arrangement, cultural characteristics and blood haemolysis. All the isolates were Gram positive cocci in cluster and were all *Staphyloccocus* the reason been the use of selective media (mannitol salt agar) which is selective for *Staphylococcus* species which is similar to the work of Bannerman [7].

Table 14 shows that 23 (46%) of the isolates were coagulase positive and 25 (50%) were coagulase negative. Based on the biochemical test, the isolates were grouped into 5 due to similarities in their biochemical characteristics which is similar to the work of Baird [14].

In Table 15, there are 2 groups of *Staphylococcus aureus* obtained. This are: C.P.S.a1 and C.P.S.a2. The C.P.S a1 i.e coagulase positive *Staphylococcus aureus* group 1 which are beta haemolysis, oxidase positive, mainly yellow mucoid raised with resistance to nitrofurantoin antibiotic. C.P.S.a2 are beta and alpha haemolysis, whitish-yellow mainly small non-mucoid to mucoid, circular, smooth with oxidase test negative and are resistance to cloxacillin antibiotic which is consistence to the work of Savic, *et al.* [15].

The coagulase negative was grouped into 3 which are: C.N.S1, C.N.S2 and C.N.S3 (coagulase negative Staphylococcus group 1, 2 and 3 respectively). C.N.S1 are gamma haemolysis, whitish yellow, oxidase positive with resistance to cefixime antibiotic. C.N.S2 are also gamma haemolysis, oxidase negative, whitish to yellowish circular colonies. C.N.S3 are alpha or beta haemolysis, oxidase positive, mucoid or non mucoid colonies with resistance to gentamicin antibiotic but susceptible to novobiocin.

The result from Table 16 shows the the antibiotic susceptibility pattern of the 5 grouped isolates. C.P.S.a1 was highly susceptible to ofloxacin, augumentine and ceftriazone but resistance to nitrofurantoin antibiotic. C.P.S.a2 are resistance to cloxacillin but highly susceptible to ofloxacin, augumentin and ceftriazone. C.N.S1 was highly susceptible to nitrofurantoin, augumentin, ceftazidime but resistance to cefixime. C.N.S2 shows no resistance to all the antibiotics used but highly susceptible to cefuroxime, ceftriazone, augumentin, nitrofurantoin, ofloxacin and cloxacillin. C.N.S3 was resistance to gentamicin and novobiocin but highly susceptible to ofloxacin, cefuroxime and ceftriazone. Out of all the antibiotics used, ofloxacin, augumentin and nitrofurantoin were very active which collaborate with the work of Akortha and Ibadin [16].

The pie chat shows the percentage or coagulase positive *Staphylococcus aureus* obtained from each town. Akungba has the highest percentage which is 14% followed by Ikun 12%, Iwaro 10% and Supare 8%. From the questionnaire 20% of the correspondents with Staphylococcus bacteriuria have been previously infected with UTI and the *Staphylococcus* bacteriuria were asymptomatic in consistence with previous investigations [17, 18].

In conclusion, female has the high risk of staphylococcal bacteriuria than male. There is an indication that sex and age largely contribute to bacteriuria. From the research, *Staphylococcus aureus* is asyptomatic in some people while partial treatment of UTI can lead to the presence of *Staphylococcus aureus* in urine which may become symptomatic when triggered by some factors. Complete treatment of Staphylococcus bacteriuria is very important as to prevent subsequent bacteriuria. The isolates were most sensitive to ofloxacin, nitrofurantoin, augumentin, cefuroxime and cetriaxone. *Staphylococcus aureus* is one of the most important opportunistic pathogen among Staphylococci causing significant infections under appropriate conditions. Although many healthy people may carry *Staphylococcus aureus* as a part of the normal microflora, however, this group of organisms becomes pathogenic in nature when conditions are favourable for it to cause tissue damage in humans and related host.

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