Umbilical Stump Colonization and Antibiotic Susceptibility Patterns of Bacteria from Umbilical Stumps of Neonates Admitted at Holy Innocents Children's Hospital, Mbarara, South Western Uganda

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Abstract

Introduction: Every year a million deaths of neonates occur worldwide due to bacteria that has ascended through the umbilical stump leading to serious infection. An umbilical stump that has just separated is known to be the best route for bacterial entrance leading to development of serious infections. It also acts as a reservoir for bacteria which potentially may gain access to the blood flow within first 2–3 days of life when the umbilical vein is still patent.

During neonatal period, the umbilical stump takes 1-2weeks to fall off under normal circumstances (WHO). Therefore understanding umbilical stump colonization is relevant within 14 days before the stump falls off. The most common isolated and recovered organisms that are known for colonization of the umbilical stump are *Staphylococcus aureus, Escherichia coli* and group B *Streptococcus*. No matter the type of colonizing bacteria, this kind of colonization normally leads to serious life-threatening infection.

Objective: The objective of this study was to determine the umbilical stump colonization and antibiotic susceptibility patterns of bacteria isolated from umbilical stumps of neonates admitted at Holy Innocents Children's Hospital.

Methods: This study used a cross-sectional study and 200 participants were enrolled from neonatal ward in the period of May-June 2022. Samples were aseptically taken off from neonates using a sterile cotton swab which were then cultured and the colonizing organisms isolated after which antimicrobial susceptibility testing was done using Kirby Bauer disk diffusion method.

Results: After culture of 200 samples, only 35 (17.5%) showed growth. Of the isolated organisms *Staphylococcus aureus* had the highest number of 15 (42.9%), this was followed by *E.coli* with 8 (22.9%), then *K.pneumoniae* at 4 (11.4%), *Proteus spp* at 4 (11.4%, then *Streptococcus spp* at 2 (5.7) and finally *Pseudomonas spp* at 2 (5.7%).

All *Staphylococcus species* were sensitive to Vancomycin whereas all *E.coli* species were resistant to Imipenem, Tetracycline, Erythromycin and Ampicillin. All *Klebsiella* and *Streptococcus* species were 100% sensitive to Ciprofloxacin, Chloramphenicol and Gentamicin. Chloramphenicol had the highest sensitivity of 85.7% followed by Gentamicin at 62.9% and Trimethoprim-sulfamethoxazole had the highest resistance of 62.9% which was followed by Tetracycline with the resistance of 54.3%. All *Streptococcus species* were sensitive to Cefoxitin, Chloramphenicol, Erythromycin and Clindamycin whereas all *Pseudomonas* species were sensitive to Ceftazidime, Piperacillin and Piperacillin-tazobactam.

Conclusion: The prevalence of umbilical stump colonisation at HICH was high at 17.5% and the main causative agents are *Staphylococcus aureus* and *Escherichia.coli*.

These organisms are resistant to commonly used antibiotics like Ciprofloxacin, Ceftriaxone and Amoxicillin-Clavulanic acid.

Background

Mortality in children less than one month is still crucial in neonatal care systems, contributing deaths of about 1.6 million per year in still developing countries (Armanian, Barekatain et al. 2020). This health concern is more serious in developing countries the fact that the mortality burden seen in the first ten days of life is estimated at about 42 percent (Armanian, Barekatain et al. 2020). An umbilical stump that has just separated is known to be the best route for bacterial entrance leading to development of serious infections (Armanian, Barekatain et al. 2020).

On the other hand, every year a million deaths of neonates occur worldwide due to bacteria that has ascended through the umbilical stump leading to serious infection. It also acts as a pool for bacteria which potentially may gain access to the blood flow within first 2–3 days of life when the umbilical vein is still patent.

This eventually leads to neonatal sepsis or simply omphalitis. However, those forms of colonization that are below the minimum infective dose (MID) mostly do not yield umbilical stump physical changes and the child in most cases will not develop neonatal sepsis.

Late onset neonatal sepsis is majorly caused by the umbilical stump colonization of bacteria following delivery. This kind of sepsis is the one that occurs after the third (3rd) up to the twentieth (28th) day of life.

During neonatal period, the umbilical stump takes 1-2weeks to fall off under normal circumstances (WHO). Therefore, understanding umbilical stump colonization is relevant within 14 days before the stump falls off.

The most common isolated and recovered organisms that are known for colonization of the umbilical stump are *Staphylococcus aureus, Escherichia coli* and group B *Streptococcus*. No matter the type of colonizing bacteria, this kind of colonization normally leads to serious life-threatening infection.

Deliveries from home, use of non-sterile delivery methods, rupturing of membranes prematurely, passage of catheters through the umbilical stump and being underweight are amongst the most common risk factors associated with umbilical stump infection (Armanian, Barekatain et al. 2020).

Worldwide it is estimated that yearly one million newborn infant's die because of bacteria that cause infection to these infants after gaining access to their bodies through the umbilical stump (Forozeshfard, Ghorbani et al. 2017).

In a study that was done in Tanzania, titled "Incidence and Risk Factors for Newborn Umbilical Stump Infections on Pemba Island, Zanzibar", of the 1653 infants that were enrolled into the study, the number of those affected ranged from 16 (1.0%, moderate to severe redness with pus discharge) to 199 (12.0%, pus and dad odor), while single signs were seen in > 20% of infants. 3 to 4 days was the median to onset of infection (Mullany, Faillace et al. 2009)

Considering other studies like in Uganda, a study that was done in western side revealed that the incidence of umbilical stump colonization was at 27.1% (Turyasiima, Nduwimana et al. 2020). This showed that there was a big problem which was mainly due to bad umbilical stump care practices and this needs to be studied and controlled.

Many cultures have different beliefs and practices as far as healing of the umbilical stump is concerned. They include; application of ashes, spice paste, butter, herbs, mud, animal lard and oils. The fact that these particles are normally polluted with spores and bacteria their use is in most cases associated with newborn skin infections caused by presence of these bacteria (Reis, de Sousa et al. 2020).

In the umbilical region there is a mixture of growth of both beneficial and harmful bacteria like clostridium tetani which causes omphalitis after getting into contact with the blood stream. Information about umbilical stump infection is still limited especially in low income countries, however it is anticipated that 2–77 per 1000 live births is the magnitude of the risk especially in hospitals with rates of mortality varying from 1–15% (Reis, de Sousa et al. 2020).

Neonatal mortality due to sepsis is known to be about 1.6 times the global number of childhood deaths due to malaria and over four times the number of childhood deaths caused by HIV. Despite its inequality burden on childhood mortality it still receives substantially less investment globally as a public health priority when compared with other major conditions (Ranjeva, Warf et al. 2018)

According to some study done in Uganda titled "Burden and factors associated with clinical neonatal sepsis in urban Uganda" it was found out that the incidence of sepsis in neonates was 11.0%. (Kayom, Mugalu et al. 2018) while in Rwanda it was found to be at 20% (INGABIRE 2018).

On the other hand, in another study that was conducted in Uganda specifically at National referral hospital Mulago, it revealed that 46 (12.8%) neonates of those that were recruited had a positive culture (Tumuhamye, Sommerfelt et al. 2020). In another study that was carried out at Mbarara Regional Referral Hospital, of the 26/80 (32.5%) neonates that were studied had positive blood cultures (Kiwanuka, Bazira et al. 2013).

Despite the high prevalence of neonatal sepsis, little information about how much umbilical stump colonization contributes to this burden is unavailable.

Furthermore, at HICH there is no microbiology laboratory for doing susceptibility testing before giving antibiotics to the neonates. Therefore, this study aimed at determining how much umbilical stump colonization contributes to the burden of neonatal sepsis in our local setting for proper management.

Materials

Study design

This study used cross-sectional design.

Study site

The study took place at Holy Innocents Children's Hospital (HICH) in Mbarara district Western Uganda.

Size determination target population

This study enrolled 200 children between 3 and 15 days of life, who were admitted at HICH

Sampling procedure

The consecutive sampling method was used whereby each participant that met the inclusion criteria would be recruited up to when the sample size was achieved.

Selection criteria

Inclusion criteria

All neonates admitted at HICH aged between 3 to 15 days were included in the study upon consent of the parent/guardian.

Exclusion criteria

- All neonates in the study age group but have anterior abdominal wall birth defects at the umbilical region. These defects included; gastroschisis and omphalocoele.
- Neonates whose parents/caretakers withdrew from the study

Sample collection and transportation

Upon consent by the parent/guardian, a sample would be taken off the neonate's umbilical stump using a sterile swab before administration of antibiotics.

The principal investigator took off each sample, with assistance from a trained research assistant.

Identification of bacteria that cause umbilical stump colonization

Isolation of bacteria:

After the samples reaching the laboratory, they would be cultured first on blood, macconkey and chocolate agars before doing a gram stain on them. This was so in order to avoid the sample getting contaminated. Cultured chocolate plates were put in anaerobic jar before incubation to cater for anaerobic organisms. The plates were then be incubated at 37^0C for about 18-24 hours. After this time the plates were checked for any significant growth and plates that showed no growth after this period were further incubated for more 24 hours before declaring absence of bacteria. Bacterial isolates were determined basing on the morphology of colonies, different biochemical tests done and gram staining (Wang, Dong et al. 2019).

Biochemical techniques for identification of isolates

Conventional biochemical techniques were used for identifying bacteria;

Catalase test

In this test the enzyme catalase catalyses breakdown of hydrogen peroxide to produce water and oxygen which is seen inform bubbles. This test helps to differentiate *Staphylococcus* organisms from other gram positive cocci (Mahmood, Hamzah et al. 2020).

Coagulase test

This test is used to differentiate between *Staphylococcus aureus* from other coagulase negative *staphylococci*. Coagulase enzyme catalyses formation of fibrin from fibrinogen when inoculated with the organism being tested. The fibrin clot normally appears in plasma after 2-3 hours and if there is no clot after this period it is further incubated up to 24 hours before declaring the test negative (Prah, Amoah et al. 2019).

Oxidase test.

It is a biochemical test that bases on enzyme production to identify enterobacteria that are non-lactose fermenting. It is used in the differentiation of *Pseudomonas*, *Proteus*, *Escherichia coli* and *Proteus*. *Pseudomonas* is the one positive (Sapkota, Timilsina et al. 2020).

Triple sugar iron agar (TSI) test

It is a biochemical test for identification of enterobacteria from other non-lactose fermenting organisms. It is attained when there is sugar fermentation with production of gas and hydrogen sulfide. It is mainly for differentiation of *Klebsiella* , *Proteus* and *E.coli* (Mahmood, Hamzah et al. 2020).

Indole test

It is a biochemical test for differentiating gram negative rods by indole production. The enteric bacteria produce an amino acid known as tryptophan that is broken down to produce indole as abi-product. Organisms are incubated in a broth for about 24 hours at 37^0C and then after to the broth Kovac's reagent is added which reacts with indole forming a bright red ring colour at the top of the broth and this is indicative of a positive test. A negative test is indicated with a no colour change after the reagent has been added to the broth. This test differentiates *E.coli* from *Klebsiella pneumoniae* (Prah, Amoah et al. 2019).

Motility test

Motility is demonstrated in such a way that motile organisms grow away from the stabbed area and circulate in the whole media whereas non-motile organisms only grow in the stabbed region. This test is used to identify *Proteus* and *E.coli* which are motile from *Klebsiella* which is non-motile (Mahmood, Abed et al. 2019).

Citrate test

Organisms containing citric acid are streaked in the citrate agar slants and butt and organisms that have citrate permease only allow intake of citric acid which leads to alkaline end products that change the pH indicator from green to blue. Isolates that change slant green are taken to be negative while those that change the slant to blue are considered positive. Generally this test is used to in the identification of enterobacteria like *E,coli* which is citrate negative and *Klesiella* which is positive (Prah, Amoah et al. 2019).

Urease biochemical test

Urea broth medium containing phenol red indicator is inoculated with isolates and organisms that produce urease decompose urea present in the medium forming ammonia as one of the bi-products. This ammonia reacts with water to form hydroxyl ions which makes the media alkaline that turn the pH indicator from orange to red-purple in colour for a positive test whereas the negative test makes the broth to keep orange colour. This test is used to differentiate enteric bacteria such as *K. pneumoniae* and *Escherichia coli which are* urease negative from *Proteus which is urease positive (Musinguzi, Kabajulizi et al. 2019).*

Bacterial antibiotic susceptibility testing

Purpose of susceptibility testing

Antimicrobial susceptibility testing is done so as to predict the likely hood of treatment success when such drug is used in vivo for therapy. Different isolated organisms are subjected to different antibiotics and the growth responses are measured (Malik, Malik et al. 2019).

Kirby Bauer disk diffusion antimicrobial susceptibility testing

The technique used here to do antimicrobial susceptibility testing was that of Kirby Bauer disk diffusion using commercially prepared antibiotic disks on MHA plates. The potentiality of antibiotics disks was assured by doing quality control weekly using *E. coli ATCC* 25922 as recommended by CSLI 2022

McFarland turbidity standard of concentration 0.5 was prepared by mixing 9.95 ml of 1 % chemically pure sulphuric acid with 0.05 ml of 1.175 % barium chloride to form a barium sulfate precipitate which brings about turbidity. Inoculums for the antimicrobial susceptibility test were compared with the standard solution for similarity.

Well isolated single colonies were transferred to the tube with sterile saline and suspensions correlated with 0.5McFarland turbidity. After the adjustment of the turbidity of the inoculum, a sterile cotton swab was immersed into the suspension and pressed firmly against the inside wall of the tube and then this was streaked over the surface of the medium thrice while turning the plate after each application to obtain an evenly distributed inoculum of the organisms and then the plate would be left at room temperature for 10 minutes to allow the agar dry (Mitchev, Allam et al. 2021).

Antimicrobial disks were made to touch on the agar plates by means of sterile forceps which were carefully pressed on the agar ensuring that contact of the two was made. Plates were then turned upside down and incubated at 37^{o}C for 24 hours. *E. coli ATCC* 25922 and *Staphylococcus aureus* ATCC 25923 were used as reference for gram negative and gram positive organisms respectively. After 24 hours of incubation, zone diameters that had complete inhibition were measured by means of a ruler and recorded in millimeter. The measuring would be done on the under surface of the plate when the lid is covered. The diameters of the zones of inhibition were measured and corresponded with the one of control organisms and then interpreted according to CLSI as sensitive, intermediate and resistant (Patel 2017).

Antibiotic discs that were used

Antibiotics	Resistant	Intermediate	Sensitive
Gentamicin	≤12	13-14	≥15mm
Nitrofurantion	≤14	15-16	≥17
Imipenem	≤19	20-22	≥23
Ceftazidime	≤21	18-20	≥21
Cefoxitin	≤21	-	≥22
Ciprofloxacin	≤15	16-20	≥21
Ceftriaxone	≤19	20-22	≥23
Co-trimoxazole(trimethoprim+ sulfamethoxazole)	≤10	11-15	≥16
Vancomycin			≥15
Piperacillin	≤17	18-20	≥21
Piperacillin-tazobactam	≤17	18-20	≥21
Ampicillin	≤13	14-16	≥17
Amoxicillin-clavulanic acid	≤13	14-17	≥18
Chloramphenicol	≤12	13-17	≥18
Tetracycline	≤14	15-18	≥19
Clindamycin	≤14	15-20	≥21

Phenotypic screening for Extended Spectrum Beta Lactamases

E.coli and Klebsiella spp that had zones of inhibition that were ≤ 22 mm were screened for ESBL through determination of their reduction in the susceptibility pattern to 3rd generation cephalosporins. Double disk synergy was used with ceftazidime disks. Ceftazidime-clavulanic and ceftazidime (30µg) were the antibiotic disks used. At a distance of 30mm these antibiotics were placed from each other. Plates were then incubated for 24hours at 37°C. 5mm enlargement in the zone of diameter for ceftazidime-clavulanic acid when compared with that of ceftazidime tested alone was the basis for determination of ESBLs as recommended by the Clinical and Laboratory Standards Institute 2020. For positive ESBL reference strain *Klebsiella pneumoniae* 700603 was used and *Escherichia coli* ATCC 25922 as an ESBL-negative reference strain (Patel 2017).

Phenotypic detection of Methicillin Resistance

Cefoxitin disc (30 µg) was used to screen for MRSA isolates by using disk diffusion technique. All isolates that had a zone of inhibition of diameter ≤ 21 mm were regarded resistant to cefoxitin. *S. aureus* ATCC 25923 strains were used as control for methicillin sensitive *S. aureus* (MSSA) and ATCC 43300 was used for methicillin resistant *S. aureus* (MRSA) as described in the CLSI guidelines (Patel 2017).

Genotypic detection of Extended Spectrum Beta Lactamases and Methicillin Resistance

The presence of Extended Spectrum Beta Lactamases genes; CTX-M, SHV and TEM was established by PCR amplification using the following primer sets; for CTX-M, Forward primer 5’-CGCTTTGCGATGTGCAG-3’ and reverse primer 5’-ACCGCGATATCGTTGGT-3’, For SHV, Forward primer 5’ATGCGTTATATTCGCCTGTG-3’ and reverse primer 5’-TGCTTTGTTATTCGGGCCAA-3’ for TEM , Forward Primer 5’-CATTTCCGTGTCGCCCTTATTC-3’ and reverse primer 5’-CGTTCATCCATAGTTGCCTGAC-3’. and Methicillin Resistance genes MECA; forward primer 5’GTGAAGATATACCAAGTGATT3’ and reverse primer 5’ATGCGCTATAGATTGAAAGGAT3’

The PCR master mix was prepared as follows: 12.5ul Hot Start Taq2x master mix (M0496S)-New England Bio-labs, 1.0µL forward (10µM), 1.0µL reverse (10µM), 5.0µL DNA template and 5.5µL RNAase-Free-H_2O making up to 25.0µL final reaction volume.

PCR amplification/Cycling

The PCR amplification was carried out in a conventional PCR Thermocycler (CLASSIC K960 Thermal Cycler), the programme was initial denaturation at 95°C for 1 minute followed by 35 cycles (denaturation at 95°C for 45 seconds, annealing at 52°C for 1 minutes and elongation at 72°C for 1 minute) and the final extension cycle of 72°C for 5 min. The annealing temperature was varying for individual genes; for SHV was 55°C, for CTX-M was 52°C, and for TEM was 55°C.

Gel electrophoresis.

DNA Amplicon was electrophoresed using 1.5% agarose gel, in 1x Tris-Borate EDTA buffer (TBE), 5µL Safe View Classic^{TM} DNA stain (cat # G108), 6x loading dye (Thermo Scientific #R0611), and DNA ladder/marker 100bp (NEB-Biolabs #N3231L). Electrophoresis was run at 200V and 80mA for 1 hour. Bands were visualized using the Gene-Flash Trans-illuminator.

3.9 Data collection

A laboratory request form was designed which helped in the capturing of demographic data, sample culture and drug susceptibility results of the participants.

Data analysis

Data was entered into Microsoft excel for cleaning and then imported into STATA software version 15•1 for data analysis.

Quality control and assurance

Quality of the results was assured and they included the following; Generally, all procedures were done while following SOPs and at all times.

At sample collection: A sterile swab would be used to avoid contamination by organisms that were not from the neonate's umbilical stump.

During transportation: Samples would be transported using a cool box with ice. Ice helps in the inactivation of growth of unwanted organisms.

During culturing: Before using the culture media quality control tests were done first and the ones that passed used leaving out those whose quality control failed.Every media would be checked for the expiry date

Ethical considerations.

Permission for conducting this study was sought Mbarara University of Science and Technology Research Ethics Committee (REC) and Holy Innocents Children's Hospital management.

Results

Demographic characteristics of study participants

Two hundred participants were enrolled for the study. Most of these participants were females (n = 114, 57%) and majority of the participants were 5 days of age and below (n=178, 89%) as shown in the table 1 below. 3 days was the mean age of the participants.

Table 1: Demographic characteristics of study participants (n = 200)

Characteristics	n	%
Gender		
Male	86	43
Female	114	57
Age distribution (days)		
1-3	109	54.5
4-6	83	41.5
7-above	08	4

Prevalence of bacterial colonization of the umbilical stump.

After culture of 200 samples from the umbilical stumps of participants, 35 (17.5%) showed growth after 48hours of incubation at 37^0C and 165 (82.5%5) did not show growth indicating the overall prevalence of bacterial colonization of 17.5% as shown in the table 2 below.

Table 2: Overall prevalence of bacterial colonisation of the umbilical stump.

Prevalence of BCU	n	%
Positive	35	17.5
Negative	165	82.5
Total	200	200

Prevalence of bacterial colonization of the umbilical stump according to gender

About gender, of the 86 (43%) male participants samples 13 (37.1%) had growth and 114 (57%) female participants only 22 (62.9%) had growth as shown in the figure 1 below.

Identification of common bacteria that colonise the umbilical stump

Bacterial isolates were 35 and they were dominated by Gram-negative bacteria 18 (51.4%) as compared to Gram positive bacteria 17 (48.6%). The highest bacterial isolates were *Staphylococcus aureus* 15 (42.9%), followed by *Escherichia coli* 8 (22.9%), *Klebsiella pneumoniae* 4 (11.4%), *Proteus spp* 4 (11.4%), *Streptococcus pneumoniae* 2 (5.7) and finally Pseudomonas *spp* 2 (5.7%) as indaicated in figure 1 below.

Phenotypic ESBL results

Isolates, *E. coli* (4) and *K. pneumoniae* (3) were resistant to Ceftazidime and were tested for ESBL as earlier indicated above. 7 of these isolates, 3 (43%) were found to be ESBL-producing whereby 2 (66.7%) were *K.pnemoniae* and 1 (33.3) *E.coli* as indicated in figure 2 below.

Gram negative genotypic screening for ESBL producing bacteria

All *Klebsiella pneumoniae* (4) and *E.coli* (8) isolates were determined for ESBL basing on the genes CTX, TEM and SHV production by using PCR amplification method and 10 (83.3%) organisms were found to be ESBL producers where by 3 (30%) were *Klebsiella pneumoniae* and 7 (70%) being *E.coli*. CTX was produced by 10 (83.3%) organisms, TEM by 9 (75%) organisms and only 1 (8.3%) organism produced SHV as shown in the table below.

Table 4: Genotypic gram negative ESBL producing bacteria (n=10)

STATUS	GENES		
	CTX	SHV	TEM
POSITIVE	10	01	09
NEGATIVE	02	11	03
PREVALENCE (%)	83.3	8.3	75

Genotypic screening of *S. aureus* for presence of mec A

All 15 *S. aureus* isolates were subjected to screening for presence of mec A gene and it was found out that only 1 (6.7%) was positive and the rest negative as shown in figure 3 below.

Bacterial susceptibility patterns to different antibiotics.

All *Staphylococcus species* were sensitive to Vancomycin whereas all *E.coli* species were resistant to Imipenem, Tetracycline, Erythromycin and Ampicillin. All *Klebsiella* and *Streptococcus* species were 100% sensitive to Ciprofloxacin, Chloramphenicol and Gentamicin. Chloramphenicol had the highest sensitivity of 85.7% followed by Gentamicin at 62.9% and Trimethoprim-sulfamethoxazole had the highest resistance of 62.9% which was followed by Tetracycline with the resistance of 54.3%.
All *Streptococcus species* were sensitive to Cefoxitin, Chloramphenicol, Erythromycin and Clindamycin whereas all *Pseudomonas* species were sensitive to Ceftazidime, Piperacillin and Piperacillin-tazobactam as shown in the table 5 below.

Table 5: showing bacterial susceptibility **patterns to different antibiotics**

Antibiotic	Pattern	S.aureus n=15	E. coli n=8	K.pneumoniae n=4	S.pnemoniae n=2	P.aeroginosa n=2	p.mirabilis n=4	Total n(%)
Cefoxitin	S	9(60)	5(62.5)	3(75)	2(100)	-	1(50)	20(57.1)
	R	6(40)	3(37.5)	1 (25)	0(0)	-	1(50)	11(31.4)
Ceftazidime	S	-	4(50)	1(25)	-	2(100)	3(75)	10(28.6)
	R	-	4(50)	3 (75)	-	0(0)	1(75)	8(22.9)
Ciprofloxacin	S	13(86.6)	2(25)	4(100)	0(0)	1(50)	3(75)	23(65.7)
	R	2(13.4)	6(75)	0 (0)	2(100)	1(50)	1(25)	12(34.3)
Piperacillin	S	-	2(25)	2(50)	-	2(100)	3(75)	9(25.7)
	R	-	6(75)	2 (50)	-	0(0)	1(25)	9(25.7)
Piperacillin-tazobactam	S	-	4(50)	3(75)	-	2(100)	2(50)	11(31.4)
	R	-	4(50)	1(25)	-	0(0)	2(50)	7(20)
Ampicillin	S	-	0(0)	1(25)	-	-	0(0)	1(2.9)
	R	-	8(100)	3(75)	-	-	4(100)	15(42.9)
Amoxicillin-clavulanate	S	-	1(12.5)	1(25)	-	-	0(0)	2(5.7)
	R	-	7(87.5)	3(75)	-	-	4(100)	14(40)
Ceftriaxone	S	-	5(62.5)	3(75)	-	-	2(50)	10(28.6)
	R	-	3(37.5)	1(25)	-	-	2(50)	6(17.1)
Erythromycin	S	11(73.3)	0(0)	1(25)	2(100)	-	3(75)	17(48.6)

	R	4(26.7)	8(100)	3(75)	0(0)	-	1(25)	16(45.7)
Chloramphenicol	S	13(86.7)	7(87.5)	4(100)	2(100)	-	4(100)	30(85.7)
	R	2(13.3)	1(12.5)	0(0)	0(0)	-	0(0)	3(8.6)
Nitrofurantoin	S	5(33.3)	7(87.5)	1(25)	-	1(50)	3(75)	17(48.6)
	R	10(66.7)	1(12.5)	3(75)	-	1(50)	1(25)	16(45.7)
Tetracycline	S	11(73.3)	0(0)	1(25)	-	0(0)	2(50)	14(40)
	R	4(26.7)	8(100)	3(75)	-	2(100)	2(50)	19(54.3)
Imipenem	S	-	0(0)	3(75)	-	1(50)	3(75)	7(20)
	R	-	8(100)	1(25)	-	1(50)	1(25)	11(31.4)
Gentamicin	S	10(66.7)	5(62.5)	4(100)	-	1(50)	2(50)	22(62.9)
	R	5(33.3)	3(37.5)	0(0)	-	1(50)	2(50)	11(31.4)
Clindamycin	S	9(60)	-	-	2(100)	-	-	11(31.4)
	R	6(40)	-	-	0(0)	-	-	6(17.1)
Trimethoprim-sulfamethoxazole	S	8(53.3)	0(0)	1(25)	1(50)	-	1(25)	11(31.4)
	R	7(46.7)	8(100)	3(75)	1(50)	-	3(75)	22(62.9)
Vancomycin	S	15(100)	-	-	-	-	-	15(42.9)
	R	0(0)	-	-	-	-	-	0(00)

Key: S-sensitive and R-Resistant

Discussion Of Results

Prevalence of bacterial colonization of the umbilical stump.

This study revealed a prevalence of 17.5% which showed similar results in a study titled “umbilical cord sepsis among neonates admitted to Mulago hospital: bacterial aetiology, prevalence and immediate outcome” that had a clinical prevalence of 17.2% (Musoke 2012). In this similar study the culture proven prevalence was 13.4% which still does not vary much from our study (Musoke 2012). In another study done in Rwanda which showed a prevalence of neonatal cord sepsis of 20% is comparable with our study results (INGABIRE 2018) and also in another study titled “Outstanding Prevalence of Methicillin Resistant *Staphylococcus aureus* in Neonatal Omphalitis” it revealed a prevalence of 21% which is also comparable to our study result (Sengupta, Banerjee et al. 2016).

This study is also similar compared to the study that was carried out in Iraq titled “Omphalitis in Neonates Admitted to Al-Ramadi Maternity and Children Hospital” that showed a prevalence of neonatal cord sepsis of 12% (Al-Ani, Johan et al. 2020).

This high prevalence of 17.5% at HICH could have been due to the fact that at the time of sample collection there were many neonates congested in one room because they had not constructed a specialize neonatal unit which could have contributed to the cross transmission of infections amongst these neonates. Secondary, there was not strict restriction of entry by caretakers who could enter in sometimes uncontrorably and this must have also contributed to the neonates cord infection.

On the other hand, this study’s outcome does not agree with other studies conducted for example in Western Uganda in a study titled “Bacteriology and Antibiotic Susceptibility Patterns among Neonates Diagnosed of Omphalitis at a Tertiary Special Care Baby Unit in Western Uganda” that showed a prevalence of neonatal omphalitis of 84.6% (Turyasiima, Nduwimana et al. 2020). However this could have been due to the fact that the sample size was smaller compared to that used in our study. In another study titled “Cord Care Practices and Omphalitis among Neonates Aged 3 - 28 Days at Pumwani Maternity Hospital, Kenya” the prevalence of omphalitis was 37.6% which is higher compared with our study. this was attributed to use of harmful substances in the process of cleaning the cord for example application of saliva by mothers as a way of cord cleaning (Kinanu, Odhiambo et al. 2016).

Identification of common bacteria that colonise the umbilical stump

In this research, the most common isolated organism was *Staphylococcus aureus* for gram positives and *E.coli* dominated the gram negative organisms followed by *Klebsiella pnemoniae* which had the same percentage with *Proteus* and then *Pseudomonas*. These results are comparable with many studies that is to say (Turyasiima, Nduwimana et al. 2020), (INGABIRE 2018), (Shah, Saxena et al. 2014), (Sawardekar 2004) which had the same organisms isolated from the umbilical cord of neonates.

The reason for isolation of such organisms could be due to the bad practices that some mothers use to clean the umbilical cords of neonates like application of saliva which has some of these organisms, use of herbs etcetera. Sometimes this could also be due to some doctors/clinicians and nurses that work on different neonates with the same gloves hence cross transmission. Also this is attributed to the fact that some care takers hold these neonates when they have not washed their hands and the fact that some of these organisms are normal flora in our hands they end up gaining access to the umbilical cords of these neonates.

Bacterial susceptibility patterns to different antibiotics

Antimicrobial resistance has become everybody’s concern across the globe. This particular research study showed that *Escherichia coli* had the most resistance pattern to majority of the commonly used antibiotics. As compared to a study done at Regional referral hospital in Mbarara the two had similar results and these findings are an indication of the burden of resistance to antibiotics around Western region of Uganda and therefore serves as a signal for combined efforts from all stake holders to work hand in hand for this burden to be combatted (Bebell, Ngonzi et al. 2017). Almost all bacterial isolates showed resistance to most of the antibiotics used to treat omphalitis and this is the same with findings in the study done by (Turyasiima, Nduwimana et al. 2020) and (Tumuhamye, Sommerfelt et al. 2022). This makes it hard for clinicians to prescribe antibiotics for neonates with omphalitis without doing susceptibility testing of antibiotics.

Most of gram negatives were resistant to Ampicillin (91.6%), Amoxicillin-clavulanic acid (87.5) and Timethoprim sulfamethoxazole (83.3%) and this is comparable with a study done by (Tumuhamye, Sommerfelt et al. 2022) where 86.8% were resistant to ampicillin, 73.7% to amoxicillin-clavulanic acid, and 60.5% to trimethoprim-sulfamethoxazole. The sensitivity of Gentamicin (82.6%),

Ceftriaxone (69.6), Ciprofloxacin (43.5%), Cotrimoxazole (00%), Ceftazidime (70%), Imipenem (100%), and Cefoxitin (57.1%) observed in a study done by (Musinguzi, Kabajulizi et al. 2019) does not agree with that of our study results of Gentamici (62.9%), Ceftriaxone (28.6), Ciprofloxacin (65.7%), Cotrimoxazole (31.4%), Ceftazidime (28.6%), Imipenem (20%) and Cefoxitin (57.1%). This high resistance could be due to the fact that there is irrational use of these antibiotics in empirical treatment of most illnesses plus using some of these drugs like C-otrimoxazole for prophylaxis in HIV infected individuals which exposes bacteria to these drugs hence leading to resistance.

Prevalence of ESBL producing genes

K. pneumoniae (66.7%) and *E. coli* (33.3%) having been the ESBL producing gram-negative bacteria in this study is in correlation with study findings by (Musinguzi, Kabajulizi et al. 2019) which also showed the same organisms to be the ESBL producers. This probably could be as a result of irrational use of third generation cephalosporins combined with other antibiotics for example gentamicin for treating most of bacterial infections plus using them for prophylaxis among neonates.

Prevalence of mecA gene

The prevalence of this gene in our study was 6.7%. Our study results does not agree with many studies for example in a study titled “Prevalence and Antibiotic Susceptibility Patterns of Clinical Isolates of Methicillin- Resistant Staphylococcus aureus in a Tertiary Care Hospital in Western Uganda” it was found out that the prevalence of mecA was 38% (Stanley, Bwanga et al. 2014). This very study had the same results as compared with a study titled “Prevalence of mecA gene among staphylococci from clinical samples of a tertiary hospital in Benin City, Nigeria” which had prevalence of mecA gene as 38.0% (Ibadin, Enabulele et al. 2017). In some other study done by (Koosha, Hosseini et al. 2016) the prevalence of mecA was 87.3%. Our study is still not comparable with a study done in Nigeria titled “Distribution of mecA gene amongst Staphylococcus aureus isolates from Southwestern Nigeria” where the prevalence of mecA was 22.2% (Alli, Ogbolu et al. 2011). This study is still not comparable with a study titled “The Prevalence of Resistance to Methicillin in Staphylococcus aureus Strains Isolated from Patients by PCR Method for Detec-tion of mecA and nuc Genes” which revealed a prevalence of 69% (Sahebnasagh, Saderi et al. 2014).

The reason for our results not comparable with other studies regarding the prevalence of mecA could be due to the fact that our sample size of the isolates was small compared with other studies which used bigger sample sizes. Another reason for the difference in these prevalences could be due to geographical locations of these studies.

Conclusion

The prevalence of umbilical stump colonisation at HICH was high at 17.5% and it is main causative agents are *Staphylococcus aureus* and *Escherichia.coli*.

These organisms are resistant to commonly used antibiotics like Ciprofloxacin, Ceftriaxone and Amoxicillin-Clavulanic acid.

Abbreviations And Acronyms

AMR Antimicrobial Resistance

ANC Antenatal Care

BCU Bacterial Colonisation of the Umbilical Stump

D.H.O District Health Officer

DST Drug Sensitivity Test

ESBL Extended Spectrum Beta Lactamase

FREC Faculty and Research and Ethics Committee

GYNAE Gynaecology

HICH Holy Innocents Children's Hospital

hr Hour

hrs Hours

ICU Intensive Care Unit

IRB Institutional Review Board

MHA Mueller Hinton Agar

MIC Minimum Inhibitory Concentration

MID Minimum Infective Dose

MMRH Mbarara Regional Referral Hospital

MRSA Methicillin Resistant Stayphylooccus aureus

MUST Mbarara University of Science and Technology

OBS Obstetrics and Gynecology

SOPs Standard Operating Procedures

WHO World Health Organisation

Declarations

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Authors' contributions

BM and FB and were involved in the conception, study design frame work and data collection. BM carried out data analysis, Presentation, interpretation and wrote first draft of the manuscript JB and FB reviewed the first draft of the manuscript.

All the author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study is available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Al-Ani, M. M., A. H. Johan, et al. (2020). "Bacterial Agents of Neonatal Septicemia in Al-Ramadi Maternity and Children Teaching Hospital." International Medical Journal **27**(4): 401-404.
2. Al-Madhoun, O. S. E.-D., A. O. A. Hasira, et al. (2020). "Low Birth Weight Prediction Using JNN." International Journal of Academic Health and Medical Research (IJAHMR) **4**(11).
3. Alli, O. T., D. Ogbolu, et al. (2011). "Distribution of mecA gene amongst Staphylococcus aureus isolates from Southwestern Nigeria." African Journal of Biomedical Research **14**(1): 9-16.
4. Armanian, A.-M., B. Barekatain, et al. (2020). "Comparison of topical breast milk versus dry cord care on umbilical cord separation, complications and cord colonization."
5. Bebell, L. M., J. Ngonzi, et al. (2017). "Antimicrobial-resistant infections among postpartum women at a Ugandan referral hospital." PLoS One **12**(4): e0175456.
6. Benova, L., M. L. Dennis, et al. (2018). "Two decades of antenatal and delivery care in Uganda: a cross-sectional study using Demographic and Health Surveys." BMC health services research **18**(1): 1-15.
7. Celebi Celik, F., F. Tuzun, et al. (2021). "Current factors affecting the risk of omphalitis in newborns: A prospective case-control study." International Journal of Clinical Practice: e14071.
8. Damian, D. J., B. Njau, et al. (2019). "Trends in maternal and neonatal mortality in South Africa: a systematic review." Systematic reviews **8**(1): 1-13.
9. Devi, R. U., S. M. Bharathi, et al. (2021). "Analysis of Young Infant Deaths Using Verbal Autopsies and Accuracy of Verbal Autopsy Tool in Chennai, India." Indian Pediatrics **58**(4): 363-366.
10. Ely, D. M. and A. K. Driscoll (2020). "Infant Mortality in the United States, 2018: Data From the Period Linked Birth/Infant Death File." National Vital Statistics Reports: From the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System **69**(7): 1-18.
11. Forozeshfard, M., R. Ghorbani, et al. (2017). "Comparison of the umbilical cord bacterial colonization in newborn infants rooming in with mothers and neonates admitted to neonatal intensive care unit." International Journal of Pediatrics **5**(11): 6009-6015.
12. Hamza, N. J. (2019). "Causes of Neonatal Mortality in Neonatal Care Units AL-Diwaniya Province–Iraq 2013." Al-Qadisiyah Medical Journal **15**(1): 76-86.
13. Ibadin, E. E., I. O. Enabulele, et al. (2017). "Prevalence of mecA gene among staphylococci from clinical samples of a tertiary hospital in Benin City, Nigeria." African health sciences **17**(4): 1000-1010.
14. INGABIRE, A. (2018). PREVALENCE AND DETERMINANTS OF UMBILICAL CORD INFECTION AMONG NEONATES IN NYARUGENGE DISTRICT, RWANDA, Mount Kenya University.
15. Kayom, V. O., J. Mugalu, et al. (2018). "Burden and factors associated with clinical neonatal sepsis in urban Uganda: a community cohort study." BMC pediatrics **18**(1): 1-8.
16. Kinanu, L., E. Odhiambo, et al. (2016). "Cord care practices and omphalitis among neonates aged 3-28 days at Pumwani Maternity Hospital, Kenya." Journal of Biosciences and Medicines **4**(01): 27.
17. Kiwanuka, J., J. Bazira, et al. (2013). "The microbial spectrum of neonatal sepsis in Uganda: recovery of culturable bacteria in mother-infant pairs." PLoS One **8**(8): e72775.
18. Koosha, R. Z., H. M. Hosseini, et al. (2016). "Distribution of tsst-1 and mecA genes in Staphylococcus aureus isolated from clinical specimens." Jundishapur journal of microbiology **9**(3).
19. Lawn, J., K. Kerber, et al. (2009). "Newborn survival in low resource settings—are we delivering?" BJOG: An International Journal of Obstetrics & Gynaecology **116**: 49-59.
20. Mahmood, A. K., K. J. Hamzah, et al. (2020). "Isolation of Escherichia Colifrom Skin Wounds in Cow." Plant Archives **20**(1): 3108-3110.
21. Mahmood, Y. S., S. M. Abed, et al. (2019). "Isolation, Identification of Bacterial Species Causing Chronic suppurative Otitis Media and Detection Some of Their Virulence Factors." Tikrit Journal of Pure Science **24**(7): 45-51.

22. Malik, M., M. I. Malik, et al. (2019). "MICROBIOLOGICAL PROFILE AND ANTIBIOGRAM OF LOWER RESPIRATORY TRACT INFECTIONS AT LAHORE GENERAL HOSPITAL, LAHORE." Journal of Postgraduate Medical Institute (Peshawar-Pakistan) **33**(1).
23. Matthews, Z., B. Rawlins, et al. (2019). Geospatial analysis for reproductive, maternal, newborn, child and adolescent health: gaps and opportunities, BMJ Specialist Journals.
24. Mitchev, N., M. Allam, et al. (2021). "Genome Sequences of Five Novel Neisseria gonorrhoeae Sequence Types Isolated in KwaZulu-Natal, South Africa." Microbiology Resource Announcements **10**(9): e01424-01420.
25. Mullany, L. C., G. L. Darmstadt, et al. (2007). "Risk factors for umbilical cord infection among newborns of southern Nepal." American journal of epidemiology **165**(2): 203-211.
26. Mullany, L. C., S. Faillace, et al. (2009). "Incidence and risk factors for newborn umbilical cord infections on Pemba Island, Zanzibar, Tanzania." The Pediatric infectious disease journal **28**(6): 503-509.
27. Musinguzi, B., I. Kabajulizi, et al. (2019). "Incidence and Etiology of Catheter Associated Urinary Tract Infection among Admitted Patients at Kabale Regional Referral Hospital, South Western Uganda." Advances in Infectious Diseases **9**(3): 183-196.
28. Musinguzi, B., I. Kabajulizi, et al. (2019). "Incidence and etiology of catheter associated urinary tract infection among admitted patients at Kabale Regional Referral Hospital, South Western Uganda."
29. Musoke, S. B. (2012). Umbilical cord sepsis among neonates admitted to Mulago Hospital: bacterial aetiology, prevalence and immediate outcome, Makerere University.
30. Nankabirwa, V., T. Tylleskär, et al. (2017). "Efficacy of umbilical cord cleansing with a single application of 4% chlorhexidine for the prevention of newborn infections in Uganda: study protocol for a randomized controlled trial." Trials **18**(1): 1-9.
31. Patel, J. B. (2017). Performance standards for antimicrobial susceptibility testing, Clinical and laboratory standards institute.
32. Prah, J. K., S. Amoah, et al. (2019). "Evaluation of urinalysis parameters and antimicrobial susceptibility of uropathogens among out-patients at University of Cape Coast Hospital." Ghana medical journal **53**(1): 44-51.
33. Ranjeva, S. L., B. C. Warf, et al. (2018). "Economic burden of neonatal sepsis in sub-Saharan Africa." BMJ global health **3**(1): e000347.
34. Reis, M. R., M. C. de Sousa, et al. (2020). "Factors Associated with Umbilical Cord Stump Healing Practices: Literature Review." Open Journal of Obstetrics and Gynecology **10**(08): 1067.
35. Sahebnasagh, R., H. Saderi, et al. (2014). "The Prevalence of Resistance to Methicillin in Staphylococcus aureus Strains Isolated from Patients by PCR Method for Detec-tion of mecA and nuc Genes." Iranian journal of public health **43**(1): 84.
36. Sapkota, A., M. Timilsina, et al. (2020). "Prevalence of urinary tract infection among school going adolescent female." MicroMedicine **8**(2): 38-45.
37. Sawardekar, K. P. (2004). "Changing spectrum of neonatal omphalitis." The Pediatric infectious disease journal **23**(1): 22-26.
38. Sengupta, M., S. Banerjee, et al. (2016). "Outstanding prevalence of methicillin resistant Staphylococcus aureus in neonatal omphalitis." Journal of clinical and diagnostic research: JCDR **10**(9): DM01.
39. Shah, D., S. Saxena, et al. (2014). "Prospective analysis of risk factors associated with group B streptococcal colonisation in neonates born at a tertiary care centre in India." Paediatrics and international child health **34**(3): 184-188.
40. Stanley, I. J., F. Bwanga, et al. (2014). "Prevalence and antibiotic susceptibility patterns of clinical isolates of methicillin-resistant Staphylococcus aureus in a tertiary care hospital in Western Uganda." British Microbiology Research Journal **4**(10): 1168-1177.
41. Tumuhamye, J., H. Sommerfelt, et al. (2020). "Neonatal sepsis at Mulago national referral hospital in Uganda: Etiology, antimicrobial resistance, associated factors and case fatality risk." PLoS One **15**(8): e0237085.
42. Tumuhamye, J., H. Sommerfelt, et al. (2022). "Umbilical Cord Stump Infections in Central Uganda: Incidence, Bacteriological Profile, and Risk Factors." International Journal of Environmental Research and Public Health **19**(23): 16055.
43. Turyasiima, M., M. Nduwimana, et al. (2020). "Neonatal umbilical cord infections: incidence, associated factors and cord care practices by nursing mothers at a tertiary Hospital in Western Uganda." Open Journal of Pediatrics **10**(02): 288.
44. Turyasiima, M., M. Nduwimana, et al. (2020). "Bacteriology and Antibiotic Susceptibility Patterns among Neonates Diagnosed of Omphalitis at a Tertiary Special Care Baby Unit in Western Uganda." International Journal of Pediatrics **2020**.
45. Turyasiima, M., M. Nduwimana, et al. (2020). "Bacteriology and Antibiotic Susceptibility Patterns among Neonates Diagnosed of Omphalitis at a Tertiary Special Care Baby Unit in Western Uganda." International Journal of Pediatrics **2020**: 4131098.

46. Wang, X., T. Dong, et al. (2019). "Isolation of bacteria capable of hydrogen production in dark fermentation and intensification of anaerobic granular sludge activity." International Journal of Hydrogen Energy **44**(30): 15853-15862.

Figures

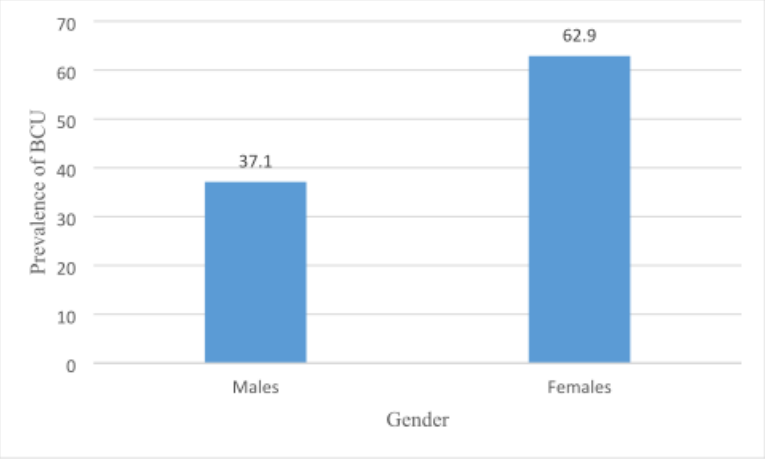

Figure 1

Prevalence of BCU according to gender.

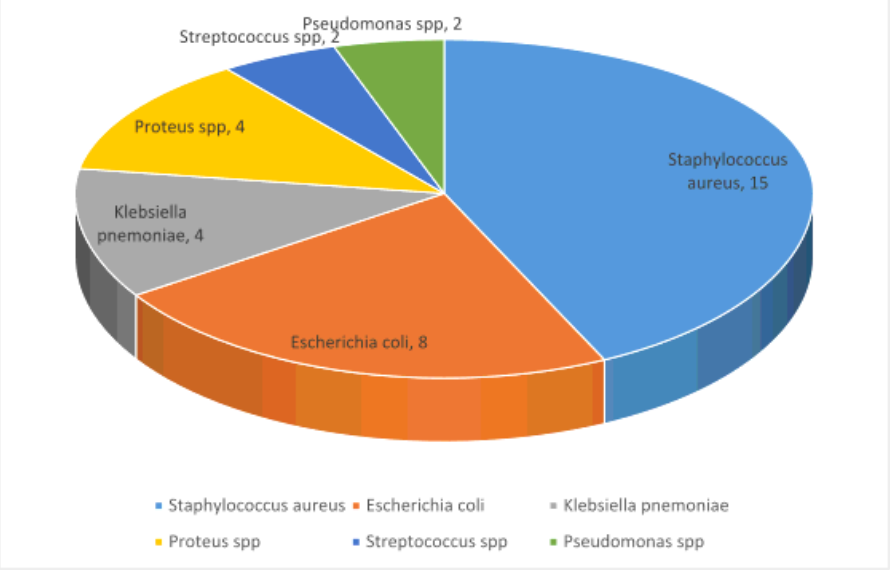

Figure 2

Bacterial isolates from the study participants (n=35)

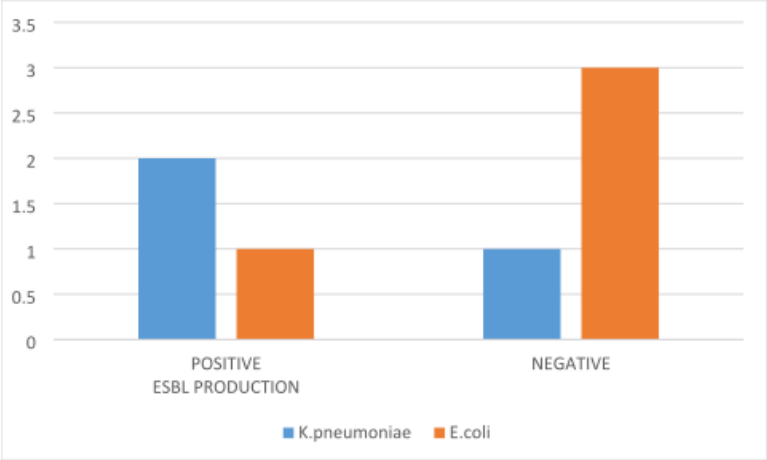

Figure 3

Gram-negative ESBL producing Bacteria (n=3)

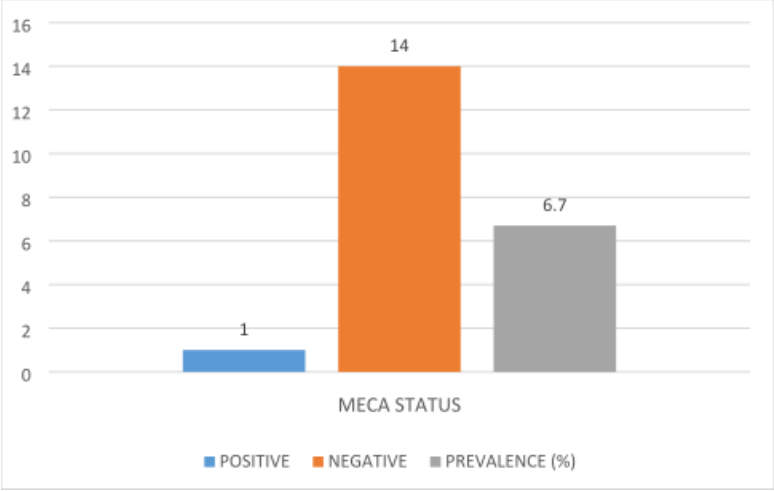

Figure 4

*Staphylococcus aureus*screening for presence of mec A (n=15)