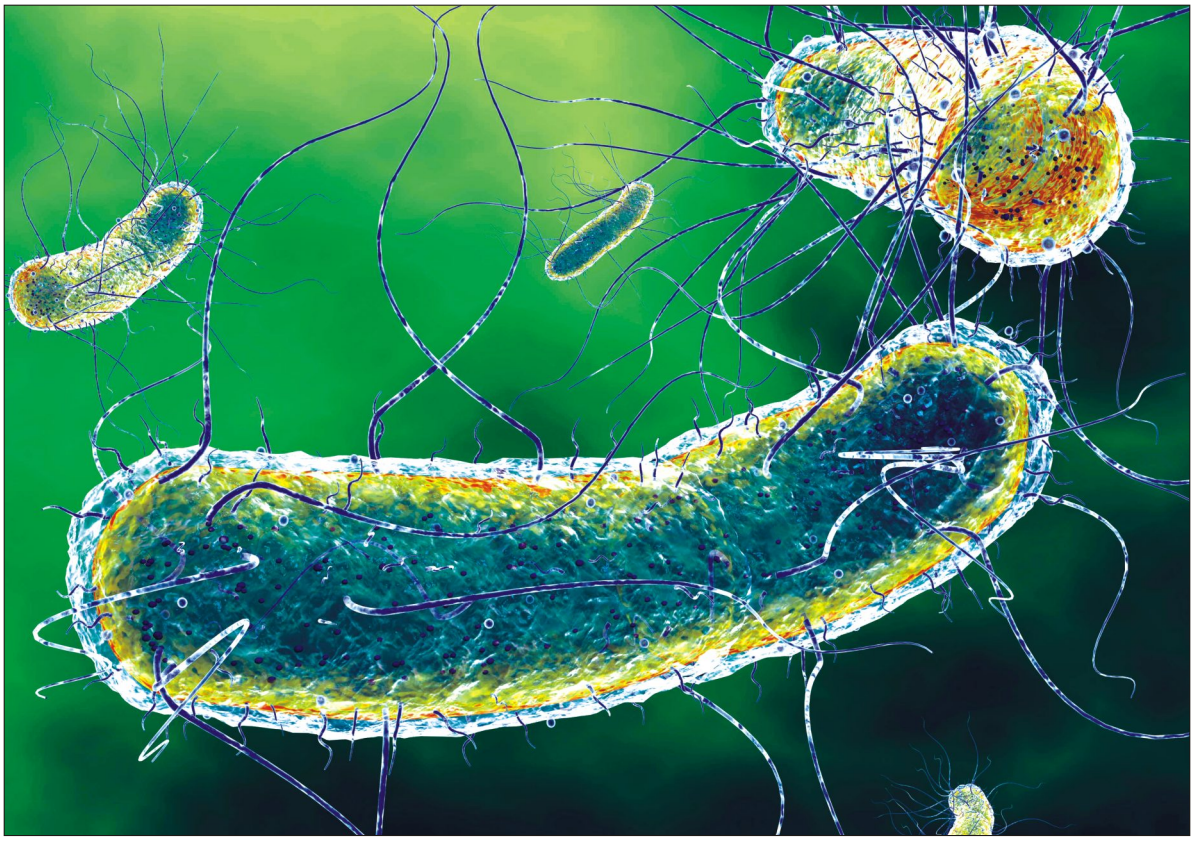

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Research Article
Antimicrobial Susceptibility Profile of Neonatal Infection and Immune Response Pattern

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Abstract

Background and Objective: Neonatal bacterial infections are considered the major causes of mortality and morbidity among neonates in developing countries. This study is aimed to study the bacterial pathogens causing neonatal infections, their antimicrobial susceptibility profile and the immune response of neonates against bacterial infection. **Materials and Methods:** About 150 samples were isolated from the neonatal intensive care unit. Bacteriological identification and susceptibility testing were done to collect samples by using the VITEK system. Collected serum samples were examined for determination of C-reactive Protein (CRP), Serum Amyloids A (SAA) and lysosomal activity. **Results:** The incidence of Gram-positive and Gram-negative organisms represented 36 and 64% respectively of culture isolates. Obtained results show many patterns of isolates and their antimicrobial susceptibility, *Klebsiella* showed a resistance rate to ampicillin of (88.9%). For *Streptococcus* all isolates were inhibited by levofloxacin. Results revealed a high level of C-reactive Protein (CRP), lysozyme and Serum Amyloids A (SAA) in the sepsis neonates group in comparison with other groups. **Conclusions:** Although bacterial infections in neonates are still manageable by the commonly used antibiotics, the development of some resistance to certain antibiotics is still a problem. Neonates bacterial infections caused elevation of some immunological parameters as SAA, CRP and Lysozyme.

Key words: Antimicrobial, infection, neonates, sensitivity, CRP, SAA, lysozyme

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Neonatal infections are acquired in utero transplacentally, intrapartum and postpartum[1]. Neonatal infections are more dangerous and more difficult to be detected than mothers and older children cause their new immune systems aren't adequately developed to fight the bacteria, viruses and parasites that cause these infections[2]. Neonatal infections are due to many bacteria such as *Escherichia coli* (*E. coli*), *Streptococcus pyogenes*, methicillin or vancomycin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA or VRSA respectively), *Klebsiella pneumonia*, *Pseudomonas aeruginosa* organisms (*P. aeruginosa*) and other infectious organisms[3].

Group B *Streptococcus* and enteric bacilli originate from the digestive system of the mother are typically identified as the cause of early-onset infections in the neonate[4]. *Listeria monocytogenes* can also cause infection and is present in the mother[5]. Infections that develop one month after infant birth is more likely due to Gram-positive bacteria and coagulase-positive Staphylococci[6]. Acquired maternal infection of *Ureaplasma urealyticum* is accompanied by a strong immune response[4,7].

Neonatal infections with *E. coli* and *S. aureus* were detected, but not as frequently as infections with Group B *Streptococcus*[8]. It is reported also that Gram-negative bacteria are the predominant causes of neonatal sepsis and among them, *K. pneumonia* is the most common pathogen, especially in developing countries[9].

There is an increase in antibiotic resistance among neonates, so continuous surveillance for antibiotic susceptibility should be done to determine the resistance pattern of bacterial isolates[10]. Resistance to ampicillin and gentamicin was detected, also significant resistance to cotrimoxazole and gentamicin among bacterial isolates. Meanwhile, *Klebsiella* and *E. coli* show concern resistance to third-generation cephalosporins[11].

Immunologic biomarkers as C-reactive Protein (CRP), lysozyme and Serum Amyloid A (SAA) have an important role in the diagnosis of early-onset neonatal sepsis and necrotising enterocolitis[12,13].

Lysozyme is a bactericidal enzyme, its levels in premature neonates were found to be significantly lower than those of matures on the first day of life. Concentrations of serum lysozyme were decreased in neonates who suffer from septicemia caused by Gram-negative organisms. Neonates levels of cord blood lysozymes were significantly lower in neonates who suffer from a predisposition to septicemia. After the first few days of life, low levels of serum lysozyme in preterm and term newborns may contribute to neonates' inability to localise an infection and destroy bacteria. Neonates with severe infection, the levels of lysozyme in serum elevated[14].

C-Reactive Protein (CRP) is used as an inflammatory marker and decision making tool for antibiotic therapy in neonates. CRP is the most widely used infection marker in neonates[15]. CRP levels rise with any source of inflammation and infection, also in neonates has been associated with prolonged labour, perinatal asphyxia, maternal pyrexia and meconium aspiration of the newborn[16].

Serum Amyloid A (SAA) is an inflammatory protein which raised during the inflammatory response to bacterial infection. SAA is an acute-phase protein that is a precursor protein in inflammation-associated reactive amyloidosis and synthesized in the liver. SAA is a prognostic marker in late-onset sepsis in preterm infants[17].

This research aimed to study bacterial pathogens which causing neonatal infections and their antimicrobial susceptibility profile. Also detection of neonates immunological response against bacterial infection.

MATERIALS AND METHODS

Study area: The study was carried out at a Women's Childbirth Hospital and King Khaled Hospital in the Hail region, from November, 2018-January, 2020.

Ethical approval: This study was approved by the institutional board member of Maternity Hospital Hail, Saudi Arabia. Neonates` parents were informed about the purpose of the medicinal analysis, study subjects were not exposed to any risks.

Collection of clinical samples: One hundred clinical samples including blood, eye swabs, nasal swabs, ear swabs, Axilla, and umbilical cords were collected from NICUs at Maternity Hospital Hail and transferred immediately to the Laboratory of Microbiology and immunology in the same hospitals for their microbiological and immunological analysis. In addition, the necessary analysis prescribed by physicians such as haemoglobin level and Complete Blood Count (CBC) were examined.

A total of 50 serum samples of neonates were included, 5 were selected in the control group (normal neonates without any signs of infection), 30 neonates were diagnosed as sepsis with positive blood culture and 15 neonates were included in the clinical sepsis group (with clinical signs of sepsis but their blood culture was negative).

Bacterial Identification and Susceptibility Testing: Collected samples were streaked by either sterile loops or swabs (ear, eye and nasal swabs) axilla and umbilical cords were also streaked on blood agar, chocolate agar, Sabouraud dextrose agar, CLED agar[18], Thioglycollate broth, Selenite F. broth, Salmonella Shigella agar and GM agar. After incubation at 37°C for 24-48 hrs, growing colonies were purified on another agar plate and slope cultures of pure isolates were made and kept throughout the experimental work[4,5,9].

Identification and susceptibility testing were done using the VITEK system. Identification of microorganisms is accomplished by biochemical methods[19].

Pure colonies were suspended in saline and were turbidometrically controlled. The suspension was inoculated into identification cards, which contain different biochemical broths in reaction cells and one negative control cell to increase viability[19].

The VITEK programmed computer determines whether each well is positive or negative by measuring light attenuation with an optical scanner[20]. When the incubation period is completed, the reactions are analysed automatically and the identification is printed[20]. Antibacterial sensitivity tests were run similarly on cards that contain dilutions of antibiotics to detect the breakpoint Minimum Inhibitory Concentration (MIC) against bacterial isolates[19]. Separate cards for Gram-negative and Gram-positive organisms were provided. The MIC cut-off values differentiating sensitive, moderate and resistant status for an organism against appropriate antimicrobials are programmed into the system[19].

Immunological parameters estimations

Lysozyme activity: Lysozyme activity was measure by using a turbidity assay in which 0.2 mg mL^{-1} lyophilized *Micrococcus lysodeikticus* in 0.04 M sodium phosphate buffer (pH 5.75) was used as substrate. 50 µL of serum was added to 2 mL of the bacterial suspension and the reduction in absorbance at 540 nm was determined after 0.5 and 4.5 min incubation at 22°C, only one unit of lysozyme activity was estimated as a reduction in absorbance of 0.001 min^{-1}. Normal sera were tested at a dilution of 1 in 5 to obtain a linear rate of clearance of the suspension[21].

Measurement of C-reactive protein: C-Reactive Protein (CRP) is considered the widest marker used for the detection of neonates bacterial infection, Using CRP to detect neonatal sepsis is hampered by its low initial sensitivity[22]. The level of C-reactive protein was determined by using an enzyme-linked immunosorbent assay kit supplied by MyBioSource, California, San Diego (USA). MBS039949 ELISA kit is based on C-reactive protein antibody and C-reactive protein antigen interactions to detect C-reactive protein antigen targets in serum samples.

Serum amyloid A (SAA): Serum amyloid A is an apo-lipoprotein synthesized by the liver[23]. Its levels rise early during bacterial inflammatory response up to 1000 times higher than the baseline of serum values but are significantly influenced by the patient's hepatic function[24].

RESULTS

The incidence of g-positive and g-negative organisms represented 36 and 64% respectively of culture isolates. *Acinetobacter baumannii* was estimated in 13% of blood samples while *Klebsiella pneumonia* was detected in 11% of samples, Meanwhile, *Klebsiella pneumoniae* was estimated in 6% among examined eye samples also *S. aureus* was detected in 5% of blood samples. *Klebsiella oxytoca* and *Enterobacter cloacae* were distributed in 2% of nasal samples, while *Acinetobacter baumannii and S. aureus* were detected in 4% of examined samples. The obtained data detect 2% of *Enterobacter gergoviae* distributed in samples isolated from Ears, while *Staphylococcus haemolyticus* was detected In one sample isolated from the axilla of examined neonates. Examined umbilical samples show the distribution of *S. aureus* in 5% of samples followed by *Klebsiella pneumoniae* 2%.

The antimicrobial profile of *Klebsiella* showed a resistance rate to ampicillin of (88.9%) and high resistance to the antibiotics used than other bacteria.

Because the antimicrobial susceptibility profile of *Staphylococcus aureus* causing neonatal infection show resistance to (fosfomycin and oxacillin). Fourteen oxacillin resistant *Staphylococcus* strains (82%) were identified, Table 1.

Acinetobacter isolates were resistant to most of the antibiotics tested, but (88.9%) of tested isolates were sensitive to colistin as represented in Table 2. *Acinetobacter baumannii* shows high resistance to used antibiotics while all *E. coli* species were resistant to amoxicillin and ampicillin, as estimated in Table 2. Meanwhile, All the *Pseudomonas aeruginosa* strains screened showed 100% resistance to ampicillin. *Enterobacteriaceae* species shows no resistant isolates to meropenem, piperacillin (combination antibiotic), and amikacin (Table 2).

Table 1: Antimicrobial susceptibility profile of *Staphylococcus* species causing neonatal infections

	Antibiotics tested MIC's and interpretation															
Isolates	Oxa	Gen	Tob	Lvx	Mxf	Ery	Lzd	Tec	Van	Tet	Tgc	Fos	Nit	Fus	Mup	Rif
S. aureus	> = 4 R	< = 0.5 S	< = 1 S	0.25 S	< = 0.25 S	> = 8 R	2 S	< = 0.5 S	16 S	< = 1 S	< = 0.12 S	16 S	32 S	N/A	N/A	< = 1 S
S. aureus (MRSA)	> = 4 R	N/A	< = 1 S	N/A	< = 0.25 S	N/A	2 S	< = 0.5 S	1 S	N/A	< = 0.5 S	1 S	< = 16S	< = 0.5	N/A	N/A
S. epidermidis	> = 4 R	< = 0.5 S	2 S	< = 0.12 S	1 S	> = 8 R	N/A	> = 32 R	> = 32R	> = 16S	-	16 S	256 R	> = 3 R	N/A	> = 32 R
S. hominis	N/A	N/A	N/A	N/A	< = .3 S	N/A	2 S	1S	N/A	N/A	< = 0.12 S	13 R	< = 2 S	> = 3 R	N/A	N/A
S. warneri	> = 4 R	> = 2 R	> = 2 R	> = 8 R	2 R	> = 8 R	1 S	> = 3	2	< = 1	< = 0.12 S	< = 8 S	< = 2	< = 3	N/A	N/A
S. pseudintermedius	> = 4 R	< = 0.5 S	< = 1 S	1 S	< = 0.25 S	> = 8 R	2 S	1 S	1 S	> = 16 R	< = 0.12 S	128 R	< = 16 S	N/A	N/A	< = 1 S
S. haemolyticus	> = 4 R	N/A	N/A	0.25 S	< = 0.3 S	N/A	N/A	4 S	1 S	N/A	0.25 S	13 R	< = 16 S	N/A	N/A	N/A

Oxa: Oxacillin, Ery: Erythromycin, Van: Vancomycin, Fos: Fosfomycin, Mup: Mupirocin, Gen: Gentamycin, Mxf: Moxifloxacin, Lzd: Linezolid, Tet: Tetracyclinee, Tec: Teicoplanin, Tgc: Tigecycline, Rif: Rifampicin, Tob: Tobramycin, Lvx: Levofloxacin, Fus: Fusidic acid, S: Sensitive, R: Resistant, N/A: Not applicable

Table 2: Antimicrobial susceptibility profile Types of *Acinetobacter* sp., *E. coli*, *Pseudomnas aeruginosa* and *Enterobacter* sp.

	Antibiotics tested MIC's and interpretation																				
Isolates	Amp	Amc	Tzp	Nfx	Ctx	Cfz	Fos	Tgc	Cfpm	Ipm	Mem	Ami	Etp	Gen	Cip	Col	Nit	Tmp	lynx	Tob	Min
A. baumanni	> = 32 R	> = 32 R	> = 13 R	> = 16 R	N/A	> = 64 R	> = 2 R	N/A	> = 64	> = 16	> = 16 R	N/A	N/A	> = 16 R	> = 4 R	< 0.5 S	>5 R	>2 R	N/A	N/A	N/A
A. lwoffi	< = 2	> = 32	> = 13 R	N/A	> = 64R	< = 1 S	N/A	N/A	> = 64 R	< = 0.25 S	> = 16 R	16 S	N/A	> = 16 R	> = 4 R	N/A	>5 R	>3 R	N/A	N/A	N/A
E. coli	8 S	4 S	< = 4 S	N/A	> = 6 R	< = 1 S	< = 2 S	N/A	< = 1 S	< = 0.5 S	< = 0.3 S	< = 2 S	<5 S	< = 1 S	< = 0.3 S	N/A	32 S	<2 S	N/A	N/A	N/A
P. aeruginosa	N/A	N/A	N/A	N/A	<= 1 S	< = 1 S	N/A	>8 R	< = 1 S	2 S	0.5 S	< = 2 S	N/A	< = 1 S	< = 0.3 S	N/A	N/A	>3 R	0.25 S	<1 S	>2 R
Enterobacter cloacae	N/A	N/A	< = 4 S	< = 0.5 S	< = 1 S	< = 1 S	64 R	N/A	< = 1 S	2 S	< = 0.3 S	< = 2 S	N/A	< = 1 S	< = 0.3 S	N/A	N/A	<2 S	N/A	N/A	N/A
Enterobacter aerogenes	N/A	N/A	< = 4 S	N/A	N/A	N/A	N/A	N/A	N/A	< = 0.3 S	< = 0.3 S	< = 2 S	N/A	< = 1 S	< = 0.3 S	N/A	N/A	< = 2 S	N/A	N/A	N/A
Enterobacter gergoviae	N/A	N/A	8 S	N/A	N/A	16 R	N/A	N/A	2 S	< = 0.3 S	0.5 S	< = 2 S	N/A	> = 2 R	> = 4 R	N/A	N/A	>3 R	N/A	N/A	N/A

Amp: Ampicillin, Amc: Amoxicllin, Tzp: Pipracillin/piperacillin, Nfx: Norfloxacin, Ctx: Cefotaxime, Cfz: Ceftazidin, Cfpm: Cefoperazone, Ipm: Imipenem, Mem: Meropenem, Ami: Amikacin, Cib: Ciprofloxacin, Col: Colistin, Nit: Nitroxolin, Tmp: Trimethoprim, Min: Minocycline

Table 3: Antimicrobial susceptibility profile of *Klebsiella* sp., *Strept. aglactia* and *Enterococcus gallinarum*

	Antibiotics tested MIC's and interpretation																				
Isolates	Amp	Amc	Tzp	Tec	Cet	Fox	Caz	Van	Cro	Cfpm	Ipm	Mem	Amk	Gen	Mox	Cip	Tgc	Nit	Tmp	Lyx	Tet	Clin
K. pneumoniae	> = 3 R	2 R	>1 R	N/A	> = 6 R	8 R	> = 6 R	N/A	> = 6 R	> = 6 R	-	> = 2 R	-	> = 2 R	N/A	0.5 S	2 S	32 S	> = 20 S	N/A	N/A	N/A
K. oxytoca	> = 3 R	8 S	<4 S	N/A	>6 R	32 R	N/A	N/A	16	-	<0.3 S	<2 S	<2 S	<1 S	N/A	<2 S	5 S	1 R	< = 20 S	N/A	N/A	N/A
S. agalactiae	N/A	N/A	N/A	<5 S	N/A	N/A	N/A	<5 S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	<1 S	<2 S	< = 1 S	1 S	>2 R	> = 8 R
E.gallinarum	N/A	N/A	N/A	<5 S	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2 R	N/A	<1 S	3 S	N/A	N/A	N/A	N/A

Clin: Clindamycin, Amk: Amikacin, Caz: Ceftazidime, Cro: Ceftriaxone

Table 4: Antimicrobial susceptibility profile of *Serratia marcescens*, *Cronobacter sakazaki*, *Raoultella ornithinolytica*, *Moraxella lacunato* and *Aeromonas salmonicida*

	Antibiotics tested MIC's and interpretation																									
Isolates	Rif	Caz	Tet	tec	Cfpm	Aza	Ami	Gen	Fox	Imp	Mox	Tob	Fos	Cip	Min	Lvx	Nit	Mem	Tgc	Col	Tmp	Amo	Etp	Amp	Tzp	Cet	Ami
S. marcescens	N/A	32 R	N/A	N/A	32 R	32 R	4 S	8 R	N/A	N/A	N/A	2 S	N/A	2 R	N/A	> = 8 R	N/A	N/A	2 S	> = 2 R	> = 3 R	N/A	N/A	N/A	N/A	N/A	N/A
C. sakazaki	N/A	< = 1 S	N/A	N/A	< = 1 S	N/A	< = 2 S	< = 1 S	< = 4 R	< = 3 S	N/A	N/A	N/A	< = 3 S	N/A	N/A	32 S	< = 2 S	1 S	N/A	< = 2 S	< = 2 S	N/A	N/A	< = 4 S	> = 6 R	N/A
R. ornithinolytica	N/A	16 R	N/A	N/A	< = 1 S	> = 6 R	< = 2 S	< = 1 S	-	< = 3 S	N/A	< = 1 S	N/A	0.5 S	4 S	N/A	N/A	< = 3 S	1 S	5 S	N/A	N/A	< = 5 S	8 S	8 S	N/A	N/A
M. lacunato	8 R	8 R	> = 3 R	N/A	N/A	N/A	N/A	N/A	N/A	< = 3 S	< = 1 S	> = 1 S	N/A	N/A	N/A	25 R	N/A	0.3 S	N/A	20 S	N/A	N/A	N/A	N/A	N/A	N/A	N/A
A. salmonicida	N/A	16 R	N/A	N/A	N/A	N/A	N/A	< = 1 S	1 S	< = 4 S	N/A	N/A	N/A	> = 4 R	N/A	N/A	N/A	N/A	N/A	N/A	> = 3 R	> = 32 R	N/A	> = 32 R	< = 4	< = 2	N/A

Aza: Azacitidine, Etb: Etophyllin, Col: Citicoline, Tec: Teicoplanin

Table 5: Immunological parameters of neonates of serum sample

Traits	Control	Sepsis (positive blood culture)	Clinical sepsis (negative blood culture)	p-value
Serum amyloid A (SAA) (μg mL^{-1})	8.85±0.23	2.56±0.69	16.45±0.38	<0.01
Lysozyme activity	0.83±0.78	1.56±0.89	0.89±0.67	<0.01
Serum CRP (mg dL^{-1})	0.365±0.78	8.384±1.36	4.183±0.765	<0.001

SAA: Serum amyloid A, CRP: C-reactive protein

Streptococcus agalactiae show high resistance to clindamycin and all isolates were inhibited by levofloxacin Table 3, while the only one isolate of Enterococcus in this study show sensitivity to all of the antibiotics tested except moxifloxacin (Table 3).

Aeromonas growth was prevented by most of the antibacterial agents, meanwhile few isolates show multidrug resistance patterns. *Aeromonas salmonicida* show resistance to Ampicillin (Table 4), while *Moraxella lacunata* was sensitive to moxifloxacin, tobramycin, trimethoprim and tigecycline as illustrated in Table 4.

Raoultella different classes of antimicrobials showed significant effects such as β-Lactam penicillins, for *Raoultella ornithine* show resistance to ceftazidime, azithromycin and levofloxacin (Table 4). While *Serratia* isolates were found to be sensitive to amikacin, tobramycin, and tigecycline, *Serratia marcescens* show sensitivity to tobramycin and amikacin (Table 4). *Cronobacter sakazakii* isolate in this study is found to be sensitive for many types of antibiotics and resistant to cephalothin as shown in Table 4.

Almost all of these bacterial strains were multidrug-resistant and have variability in their sensitivity or resistant profiles, no strain was either completely resistant or sensitive to a certain antibiotic. Such types of studies with good infection control practice and the use of sensible antibiotics will guarantee the success of infection management and maintain the potency of available antibiotics.

The mean of CRP in the control group was 0.365±0.78 mg dL^{-1} and the sepsis group showed a higher value of CRP than clinical sepsis and control groups with a mean of 8.384±1.36 mg dL^{-1}. CRP results revealed a significant increase in the sepsis group in comparison with control and clinical sepsis as shown in (Table 5). Obtained results revealed a high concentration of lysozyme in the sepsis group in comparison with the control group. The mean plasma lysozyme concentration in healthy neonates was 0.83±0.78 μg mL^{-1} while in sepsis neonates (positive blood culture) was 1.56±0.89 μg mL^{-1} (Table 5).

SAA concentration in serum reported high concentration in the sepsis group in comparison with other groups. The mean of SAA in the control group was 8.85±0.23 μg mL^{-1}, while the sepsis group showed a higher value of SAA with a mean of 23.56±0.69 μg mL^{-1} (Table 5).

DISCUSSION

Antimicrobial susceptibility testing show variable levels of resistance to tested antibiotics. *Klebsiella* species isolates have high rates of resistance to the used antimicrobial agents[20]. Most *K. pneumoniae* isolates are resistant to amoxicillin and ampicillin, due to a constitutively expressed chromosomal class-A β-lactamase[20]. Our study showed a resistance rate to ampicillin of (88.9%). However, *Klebsiella* isolates were reported to be sensitive to fluoroquinolones. Ciprofloxacin, an orally well-absorbed quinolone, is commonly used for empirical UTI treatment[20]. Our study showed a sensitivity rate to Ciprofloxacin of (95%). Because of fail treatment with routine drugs fluoroquinolones have been used as an alternative medication.

Methicillin-resistant *S. aureus* (MRSA) is a major nosocomial pathogen causing serious morbidity and mortality in immunosuppressed patients[25]. Using broad-spectrum antibiotics in treatment protocol also increases the risk of acquiring resistant bacteria and MRSA. In our study one case of MRSA was reported, treatment antibiotic-resistant bacteria is a therapeutic problem. Most of the *Staphylococcal* strains are reported to be resistant to oxacillin. Because of inactivation of antibiotic as a result of structural modification by enzymatic action, prevention of access to a target by altering outer membrane permeability, alteration of an antibiotic target site, efflux pump which pumps out antibiotic and target enzyme bypass[26]. This study identified fourteen oxacillin resistant *Staphylococcus* strains (82%), on the other hand, a kind of *S. aureus* known as hetero-VRSA, frequently produces VRSA when exposed to vancomycin and is linked to infections. The presence of hetero-VRSA is thought to be a good predictor of vancomycin's therapeutic success in hospitals. Vancomycin resistance is acquired when the cell wall thickens as a result of the accumulation of significant amounts of peptidoglycan. For all isolated VRSA strains, this appears to be a common resistance mechanism[27].

In this study 3 cases (15%) of VRSA were identified, a recent report of *Staphylococcus* resistance to commonly

used antibiotics highlights the importance of the development of new agents such as tigecycline for adequate treatment of highly resistant strains. This research also evaluates tigecycline activity against clinically isolated *Staphylococcus* species.

Similar to the results of other studies[28], tigecycline was effective against all of *Staphylococcus* species, there was no resistance to tigecycline among Staphylococcal isolates in biologic samples obtained in this study.

A. baumannii causes hospital-acquired epidemics as a result of treatment failures caused by multiple antibiotic resistances[29]. Colistin remains one of the last-resort antibiotics for the treatment of multidrug-resistant *Acinetobacter*[30]. In this study, *Acinetobacter* isolates were resistant to most of the antibiotics tested, but (88.9%) of tested isolates were sensitive to colistin.

In this study isolated *E. coli* were resistant to ampicillin and amoxicillin, indicating a cautious use of these antibiotics for the treatment of *E. coli* infections. *E. coli* resistance to penicillins is increasing by the day, however, there are only a few studies that show 100% resistance to penicillins[31].

Antibiotics active against *E. coli* were amikacin, Imipenem, meropenem generally with no resistant isolates. This is also reported in other studies[31]. It is recommended to treat the UTIs caused by *E. coli* by combination therapy especially amikacin and ciprofloxacin to provide better results[32].

Because of the synergy between a multi-drug efflux system or a type 1 AmpC-lactamase and limited outer membrane permeability, *P. aeruginosa* is naturally resistant to numerous antimicrobial agents[33]. Almost all of the six *P. aeruginosa* strains tested were ampicillin-resistant 100% of the time. For many Gram-positive and Gram-negative bacteria, carbapenems (such as Meropenem and Imipenem) are the medications of choice.

The most efficacious antibiotics that we found in our study were Carbapenems. In this study, there was no resistance to Impineme and Meropenem. Similar results have been published in various other studies too indicating that Carbapenems are the drugs of choice in case of infections, especially multidrug-resistant *P. aeruginosa* with minimum detected resistance[34]. Obtained results show no resistance for ciprofloxacin and levofloxacin, while this activity was reported in other studies[35]. Although both ciprofloxacin and levofloxacin are active against *P. aeruginosa*, levofloxacin use might be associated with a higher risk of isolation of quinolone-resistant *P. aeruginosa* than ciprofloxacin[35].

Enterobacteriaceae show resistance to monotherapy of cephalosporins and penicillins. A combination of ampicillin, amoxicillin and third-generation cephalosporins with sulbactam and monotherapy of amikacin showed higher sensitivity to *Enterobacteriaceae* infections but maximum sensitivity was shown by carbapenems[36]. The obtained results revealed, no resistant isolates to meropenem, piperacillin (a combination antibiotic) and amikacin.

Levofloxacin inhibited all *Streptococcus* isolates, and time-kill data from additional investigations showed that levofloxacin is bactericidal against most Streptococci and had increased action when coupled with gentamicin. Levofloxacin, alone or in combination with an aminoglycoside, could be a good alternative to more traditional treatments for common or serious streptococcal infections[37].

On the other hand, most Streptococci show similar susceptibility patterns to the majority of antibiotics. They remain uniformly sensitive to vancomycin, teicoplanin, trimethoprim, chloramphenicol and rifampicin. In this study, all of the isolates were trimethoprim sensitive[38]. The only isolate of enterococcus in this study show sensitivity to all of the antibiotics tested except moxifloxacin. This has been described in other studies as Fluoroquinolone resistant species[39].

Antimicrobial resistance of *Aeromonas* species is commonly chromosomally mediated, however, β-lactamases produced via way of means of aeromonads might also additionally on occasion be encoded via way of means of plasmids or integrons[40].

Aeromonas isolate was inhibited by most antimicrobial agents, with few isolates showing a multidrug resistance profile. It shows resistance to ampicillin, amoxicillin and ceftazidime as reported by *Murray et al.*[41]. Most of *Moraxella* species except *Moraxella catarrhalis*, are susceptible to penicillin, cephalosporins, tetracyclines, quinolones, and aminoglycosides[42]. In our study, *Moraxella* isolates were sensitive to trimethoprim, moxifloxacin, tobramycin, and tigecycline.

Raoultella ornithinolytica causes enteric fever, a different class of antimicrobials showed significant effects such as β-Lactam penicillins (amoxicillin/clavulanic acid, ampicillin/sulbactam and piperacillin), cephalosporins (cefazolin, ceftriaxone and cefuroxime), monobactam (aztreonam), fluoroquinolones (ciprofloxacin and levofloxacin), aminoglycosides (amikacin and tobramycin) and tetracycline[43]. This sensitivity pattern appears also in our results. *Serratia* isolate was found to be sensitive to amikacin, tobramycin

and tigecycline. Amikacin is useful in treating patients infected with gentamicin-resistant *S. marcescens* organisms. The capacity of *S. marcescens* strains to develop resistance to amikacin limits the usefulness of this antibiotic in the treatment of deep tissue infections[44]. *Cronobacter sakazakii* isolate in this study is found to be sensitive for many types of antibiotics that are also reported in other studies, such infections are treated with ampicillin and gentamicin[45]. *Enterobacter* species are resistant to narrow-spectrum penicillins which have good activity against *E. coli*. Increasing resistance of Cronobacter to antibiotics should prompt researchers to consider carbapenems in concert with an aminoglycoside. Minimizing the use of broad-spectrum antibiotics and selecting antibiotics on basis of sensitivity results are of paramount importance[45].

CRP is one of every of the foremost used laboratory tests for neonatal bacterial infection and despite the continuing emergence of the latest infection markers[15]. CRP incorporates a role within the diagnosis of early-onset neonate sepsis and there's an association between CRP levels and sepsis[46]. An association between high CRP levels and neonatal sepsis has been detected, despite CRP may be a non-specific marker in inflammatory reactions, the relatively high specificity and sensitivity above 4.09 ng mL^{-1} level of CRP strengthen the use of CRP within the diagnosis of neonatal sepsis[47].

Lysozyme is considered an indicator of innate immune response and phagocytic activity. This is based on, phagocytosis is stimulated by the presence of an antigen, the amount of serum lysozyme is increased[21].

SAA show a significant increase in the sepsis group in comparison with another group, this was contradictory to the study done by who reported that sepsis produced an elevation of SAA levels than what occurred with the control normal group. There is a high probability of neonates with normal SAA levels when there is no neonatal sepsis, neonate with symptoms of sepsis will have blood culture-positive neonatal sepsis if SAA levels is $\geq$10 μg mL^{-1} [48].

The findings of these studies suggest that CRP, Lysozyme and SAA, are visiting be helpful as diagnostic and prognostic markers of neonatal sepsis in routine clinical settings. However, it's recommended to check the diagnostic efficiency of CRP during a combination with other chemical markers to extend the specificity of the test.

Overall, neonatal septicemia is also a life-threatening emergency and its rapid treatment with antibiotics is very important. The knowledge of the etiological organisms of neonatal sepsis and their antibiotic susceptibility profile is critical for effective therapeutic intervention. Thus, minimizing the use of broad-spectrum antibiotics and selecting antibiotics on basis of sensitivity results are of paramount importance. This study has enrolled neonates which were only admitted to Women's Childbirth Hospital and King Khaled Hospital in the Hail region Future research should cover suspected neonates from different regions.

CONCLUSION

We conclude that a Higher proportion of the neonates with sepsis showed raised CRP, SAA and lysozyme levels than those without sepsis and the level correlated well to the severity of the condition. The findings of this study suggest that CRP, SAA and lysozyme can be used as diagnostic and prognostic biomarkers of neonatal sepsis.

SIGNIFICANCE STATEMENT

This study discovers that CRP, SAA and lysozyme as biomarkers of neonatal sepsis. Also, it will help the researcher for antibiotics treatment protocols of neonates, through the determination of antimicrobial sensitivity patterns and immune responses developed.

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REFERENCES

1. Bale, J.R., B.J. Stoll and A.O. Lucas, 2003. Improving Birth Outcomes: Meeting the Challenge in the Developing World. Washington, DC: The National Academies Press.
2. Sharma, A., A. Thakur, C. Bhardwaj, N. Kler, P. Garg, M. Singh and S. Choudhury, 2020. Potential biomarkers for diagnosing neonatal sepsis. Curr. Med. Res. Pract., 10: 12-17.
3. Hornik, C.P., P. Fort, R.H. Clark, K. Watt and D.K. Benjamin *et al*., 2012. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum. Dev., 88: S69-S74.
4. Shet, A. and P. Ferrieri, 2004. Neonatal and maternal group B streptococcal infections: A comprehensive review. Indian J. Med. Res., 120: 141-150.
5. Janakiraman, V., 2008. Listeriosis in pregnancy: Diagnosis, treatment, and prevention. Rev. Obstet Gynecol., 1: 179-185.

6. Wormser, G.P. and J. Goldfarb, 2001. Infectious diseases of the fetus and newborn. Clin. Infect. Dis., 33: 417-418.
7. Pryhuber, G.S., 2015. Postnatal infections and immunology affecting chronic lung disease of prematurity. Clin. Perinatol., 42: 697-718.
8. Chan, G.J., A.C. Lee, A.H. Baqui, J. Tan and R.E. Black, 2013. Risk of early-onset neonatal infection with maternal infection or colonization: A global systematic review and meta-analysis. PLoS Med., Vol. 10. 10.1371/journal.pmed.1001502.
9. Zakariya, B.P., V.B. B, B.N. Harish, T.A. Babu and N.M. Joseph, 2012. Risk factors and predictors of mortality in culture proven neonatal sepsis. Indian J. Pediatr.s, 79: 358-361.
10. Shah, A.J., S.A. Mulla and S.B. Revdiwala, 2012. Neonatal sepsis: High antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit of a tertiary care hospital. J. Clin. Neonatol., 1: 72-75.
11. Thaver, D., S.A. Ali and A.K.M. Zaidi, 2009. Antimicrobial resistance among neonatal pathogens in developing countries. Pediatr. Infect. Dis. J., 28: S19-S21.
12. Memar, M.Y., N. Alizadeh, M. Varshochi and H.S. Kafil, 2017. Immunologic biomarkers for diagnostic of early-onset neonatal sepsis. J. Maternal-Fetal Neonatal Med., 32: 143-153.
13. Ng, P.C., T.P.Y. Ma and H.S. Lam, 2015. The use of laboratory biomarkers for surveillance, diagnosis and prediction of clinical outcomes in neonatal sepsis and necrotising enterocolitis. Arch. Dis. Childhood Fetal Neonatal Edition, 100: F448-F452.
14. Galask, R.P., B. Larsen and M.J. Ohm, 2009. Infection in maternal-fetal medicine: An overview. Global Library Women`s Med., Vol. 2009. 10.3843/glowm.10173.
15. Ismail, A.Q.T. and A. Gandhi, 2014. Using crp in neonatal practice. J. Maternal-Fetal Neonatal Med., 28: 3-6.
16. Rifai, N. and P.M. Ridker, 2003. Population distributions of C-reactive protein in apparently healthy men and women in the United States: Implication for clinical interpretation. Clin. Chem., 49: 666-669.
17. Arnon, S., I. Litmanovitz, R. Regev, S. Bauer, M. Lis, R. Shainkin-Kestenbaum and T. Dolfin, 2005. Serum amyloid A protein is a useful inflammatory marker during late-onset sepsis in preterm infants. Neonatology, 87: 105-110.
18. Reller, L.B., M. Weinstein, J.H. Jorgensen and M.J. Ferraro, 2009. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clin. Infect. Dis., 49: 1749-1755.
19. Ligozzi, M., C. Bernini, M.G. Bonora, M. de Fatima, J. Zuliani and R. Fontana, 2002. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J. Clin. Microbiol., 40: 1681-1686.
20. Montiel, N., M. Escalona, Y. Carrero, K.E. Urdaneta, M.A. Castillo, D. Martínez-Méndez and N. Semprún-Hernández, 2018. Increased serum lysozyme activity in children with autism spectrum disorder. Invest. Clín., 59: 250-259.
21. Chirico, G. and C. Loda, 2011. Laboratory aid to the diagnosis and therapy of infection in the neonate. Pediatr. Rep., Vol. 3. 10.4081/pr.2011.e1.
22. Stanciu, G.D., A. Luca, R.N. Rusu, V. Bild and S.I.B. Chiriac *et al*., 2020. Alzheimer's disease pharmacotherapy in relation to cholinergic system involvement. Biomolecules, Vol. 10. 10.3390/biom10010040.
23. Chauhan, N., S. Tiwari and U. Jain, 2017. Potential biomarkers for effective screening of neonatal sepsis infections: An overview. Microb. Pathog., 107: 234-242.
24. El-Bouamri, M.C., L. Arsalane, Y. El-Kamouni and S. Zouhair, 2015. Antimicrobial susceptibility of urinary *Klebsiella pneumoniae* and the emergence of carbapenem-resistant strains: A retrospective study from a university hospital in Morocco, North Africa. Afr. J. Urol., 21: 36-40.
25. Kitara, L.D., A.D. Anywar, D. Acullu, E. Odongo-Aginya, J. Aloyo and M. Fendu, 2011. Antibiotic susceptibility of *Staphylococcus aureus* in suppurative lesions in Lacor Hospital, Uganda. Afr. Health Sci., 11: S34-S39.
26. Chakraborty, S.P., S.K. Mahapatra and S. Roy, 2011. Biochemical characters and antibiotic susceptibility of *Staphylococcus aureus* isolates. Asian Pac. J. Trop. Biomed., 1: 212-216.
27. Hiramatsu, K., 2001. Vancomycin-resistant *Staphylococcus aureus*: A new model of antibiotic resistance. Lancet Infect. Dis., 1: 147-155.
28. Khalili, H., S. Dashti-Khavidaki, S. Khaleghi, Z. Maleki and M. Rasoolinejad, 2010. Evaluation of tigecycline activity against methicillin-resistante *Staphylococcus aureus* isolated from biological samples. Iran. J. Pharmaceut. Res., 9: 61-65.
29. Altun, H.U., S. Yagci, C. Bulut, H. Sahin, S. Kinikli, A.K. Adiloglu and A.P. Demiroz, 2014. Antimicrobial susceptibilities of clinical *Acinetobacter baumannii* isolates with different genotypes. Jundishapur J. Microbiol., Vol. 7. 10.5812/jjm.13347.
30. Polin, R.A., 2012. Management of neonates with suspected or proven early-onset bacterial sepsis. Pediatrics, 129: 1006-1015.
31. Falagas, M.E., A.P. Grammatikos and A. Michalopoulos, 2008. Potential of old-generation antibiotics to address current need for new antibiotics. Exp. Rev. Anti-infective Ther., 6: 593-600.
32. Sabir, S., A.A. Anjum, T. Ijaz, M.A. Ali, M.U.R. Khan and M. Nawaz, 2014. Isolation and antibiotic susceptibility of *E. coli* from urinary tract infections in a tertiary care hospital. Pak. J. Med. Sci., 30: 389-392.

33. Sivanmaliappan, T.S. and M. Sevanan, 2011. Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* from diabetes patients with foot ulcers. Int. J. Microbiol., Vol. 2011. 10.1155/2011/605195.
34. Abbas, S.H., M.Z.I. Khan, M. Naeem, M. Adil, S.M. Naz, A. Khan and M.U. Khan, 2015. Sensitivity of patterns of *Pseudomonas aeruginosa* isolates obtained from clinical specimens in Peshawar. J. Ayub Med. Coll. Abbottabad, 27: 329-332.
35. Kanj, S.S. and Z.A. Kanafani, 2011. Current concepts in antimicrobial therapy against resistant gram-negative organisms: Extended-spectrum β-lactamase–producing enterobacteriaceae, carbapenem-resistant enterobacteriaceae and multidrug-resistant *Pseudomonas aeruginosa*. Mayo Clinic Proc., 86: 250-259.
36. Summaiya, M., C. Jaykaran and P. Tanvi, 2011. Antibiotic sensitivity of enterobacteriaceae at a tertiary care center in India. Chron. Young Sci., 2: 214-218.
37. Garrison, M.W., 2003. Comparative antimicrobial activity of levofloxacin and ciprofloxacin against *Streptococcus pneumoniae*. J. Antimicrob. Chemother., 52: 503-506.
38. Grinwis, M.E., C.D. Sibley, M.D. Parkins, C.S. Eshaghurshan, H.R. Rabin and M.G. Surette, 2010. Macrolide and clindamycin resistance in *Streptococcus milleri* group isolates from the airways of cystic fibrosis patients. Antimicrob. Agents Chemother., 54: 2823-2829.
39. Ramos, S., V. Silva, M.D.L.E. Dapkevicius, G. Igrejas and P. Poeta, 2020. Enterococci, from harmless bacteria to a pathogen. Microorganisms, Vol. 8. 10.3390/micro-organisms8081118.
40. Aravena-Román, M., T.J.J. Inglis, B. Henderson, T.V. Riley and B.J. Chang, 2012. Antimicrobial susceptibilities of *Aeromonas* strains isolated from clinical and environmental sources to 26 antimicrobial agents. Antimicrob. Agents Chemother., 56: 1110-1112.
41. Murray, P.R. and E.J. Baron, 2007. Manual of Clinical Microbiology. 9th Edn., ASM Press, Washington, DC., ISBN: 1555813712, pp: 133-151.
42. Trivedi, M.K., S. Patil, S. Harish, M. Gangwar and S. Jana, 2015. Biofield treatment: An alternative approach to combat multidrug-resistant susceptibility pattern of *Raoultella ornithinolytica*. Altern. Integr. Med., Vol. 4. 10.4172/2327-5162.1000193.
43. Mahlen, S.D., 2011. *Serratia* infections: From military experiments to current practice. Clin. Microbiol. Rev., 24: 755-791.
44. Hunter, C.J., M. Petrosyan, H.R. Ford and N.V. Prasadarao, 2008. *Enterobacter sakazakii*: An emerging pathogen in infants and neonates. Surg. Infect., 9: 533-539.
45. Mathai, E., U. Christopher, M. Mathai, A.K. Jana, D. Rose and S. Bergstrom, 2004. Is C-reactive protein level useful in differentiating infected from uninfected neonates among those at risk of infection? Indian Pediatr., 41: 895-900.
46. Fattah, M.A., S.A. Alaskar, R.I. Alkhamis, F. Othman and T. Karar, 2019. The association between neonatal sepsis and CRP a cross sectional study at tertiary care hospital. Int. J. Med. Res. Health Sci., 8: 54-60.
47. Elmashad, G.M., H.M. Elsayed, Z.A. Omar, E.A. Badr and O.M. Omran, 2019. Evaluation of serum amyloid A protein as a marker in neonatal sepsis. Menoufia Med. J., 32: 1094-1098.
48. Krishnaveni, P., M.N.V. Gowda and G.C.M. Pradeep, 2016. Estimation of serum amyloid A protein in neonatal sepsis: A prospective study Int. J. Med. Sci. Public Health, 5: 1665-1672.