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Staphylococcus Aureus Nasal Colonization in the Neonatal Intensive Care Unit of Beni-Suef University Hospital

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Abstract

Staphylococcus aureus (S. aureus) remains one of the most frequently occurring community-acquired as well as hospital-acquired pathogens with high rates of hospital-acquired infections. S.aureus is an important pathogenic bacterium. It resides predominantly in the anterior nares, and extra-nasal sites including the skin, perineum, and pharynx, and less frequent in the gastrointestinal tract and the vagina. About 30% of the general population is nasal carriers of the bacterium. Infections caused by this organism could be exogenous or endogenous in origin, and they include impetigo, cellulitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), septicemia, and pneumonia. Healthcare-associated infections by S. aureus are about 20% worldwide. S. aureus infection at NICU remains a major health challenge as colonized healthcare workers, patients, and contaminated surfaces serve as reservoir for infection. The incidence of healthcare-associated infections caused by S. aureus continues to increase worldwide. The goal of this study is to assess the nasal carriage of S. aureus isolated from neonates and health care workers (HCWs) in the NICU of Beni Suef University Hospital. Also, our study provides valuable insights documenting important factors that affect susceptibility and outcomes of neonatal sepsis which is a global public health issue.

Keywords: Nasal S. aureus carrier, NICU, HCWs, Neonatal sepsis.

1. Introduction

Neonatal sepsis is one of the major causes of morbidity and mortality among the newborns in the developing world. It is characterized by systemic inflammation and general damage of tissues. It can be diagnosed on the basis of clinical or microbiological data**.** According to the levels and trends in Child Mortality Report (2019), a large

variation of neonatal mortality rates exists between different regions worldwide. The highest neonatal mortality rates are reported in developing countries in Sub-Saharan Africa and south Asia [1].

In a systematic review assessing the global burden of sepsis among neonates and children, it was reported that mortality due to severe sepsis can reach 20% of affected cases [2].

Sepsis during the neonatal period can lead to various complications. The short-term complications include respiratory failure, pulmonary hypertension, cardiac failure, shock, renal failure, liver dysfunction, and cerebral edema. Some long-term complications include developmental delays and sensory and neurological dysfunction [3].

The assessment of the prevalence, clinical outcomes, and risk factors for neonatal sepsis among neonates will help provide information to policymakers and/or management to take more preventive measures in reducing the risk among neonates. Furthermore, the knowledge of pathogens causing infections in young infants is essential for designing hospital-based management strategies [4].

The organisms most frequently involved in early-onset neonatal sepsis of term and preterm infants together are Group *A beta haemolytic streptococci* (GBS) and *E.coli*, which account for approximately 70% of infections combined. Additional pathogens to consider, which account for the remaining minority of cases, are other streptococci (most commonly viridans group streptococci but also *Streptococcus pneumoniae) , S.aureus, Enterococcus* spp., Gram-negative enteric bacilli such as *Enterobacter spp., and Listeria monocytogenes* [5].

A persistent carriage is more common in children than in adults, and many people change their pattern of carriage between the age of 10 and 20 years. The reasons for these differences in the colonization patterns are unknown. A persistent carriage seems to have a protective effect on the acquisition of other strains, at least during the hospitalization of the patients. This barrier to the colonization is reduced when the carriers are treated with antibiotics [6].

Healthcare Workers (HCWs) constitute an important reservoir of *S.aureus*. Several studies have reported that the rate of the nasal carriage of S.aureus among the HCWs ranges from 16.8% - 56.1 %. So, screening of the nasal carriage in HCWs is an important component in the control of infections in any health care facility. The identification of the colonized members allows the appropriate management of these persons, to prevent the spread to others [7].

2. Patients and Methods

This was a Cross- sectional study that was carried out at the Neonatal Intensive Care Unit (NICU) of Beni-Suef University Hospital over a period of six months (April 2020 to September 2020). The unit admits inborn neonates from the obstetrics

department, Private Hospital and Health Centers which averages 40,000 deliveries per year. The NICU has a capacity of 25 incubators with a nurse : patient ratio of 1:2. The population for the study comprised of 330 neonates admitted to NICU at Beni-Suef University Hospital in this period. A total of about average 24 Health care workers (HCWs) willingly accepted to participate in the study monthly, none of the participants reported complications or infections involving the ear, nose or throat.

2.1 Inclusion criteria:

1- All neonates admitted to NICU of Beni-Suef University Hospital in this period, a 2nd sample for neonate was on the after 4th -7th day of admission or at discharge.

2- HCWs (doctors & nurses) samples were taken monthly.

A. **History:** Detailed perinatal history of the neonates: including gestational age, mode of delivery, early postnatal cyanosis, jaundice or convulsions, risk factors including (chest tube insertion, endotracheal intubation and surgical procedures)

B. Examination: i. Gestational age, birth weight and gender.

ii. General and systemic examinations were done including: vital signs (heart rate, respiratory rate, temperature and blood pressure using the DINA MAP), neonatal reflexes (e.g. Moro's and suckling reflexes).

C. Investigations: Complete blood count (CBC). reactive protein (CRP),

Nasal swabs were taken from both neonates and health care workers: Two samples were taken from neonates; the first one at admission while the second swab was taken after 4th -7th day of admission or at discharge.

HCWs (doctors & nurses) samples were taken monthly.

From each neonate or medical personnel, one swab from anterior nares using sterile cotton swabs.

The nasal swabs were inoculated on Blood agar plate and and Mannitol salt agar (MSA) plate (oxoid, USA), incubated at 37 °C for 18 to 24 *S. aureus* colonies appear yellowish suggesting fermentation of mannitol on MSA plates after 18 to 24 h of incubation. Purity plating was performed on culture plates with mixed bacteria growth to obtain pure discrete colonies. Following purity plating, Gram's staining was performed to establish the Gram reaction of the cultures as described previously **(CLSI ., 2019).** **Catalase test** was done on all colonies (test organism) confirmed as Gram positive cocci to separate staphylococci from streptococci. A colony from purity plating was added to a drop of 3% hydrogen peroxide (3% H_2O_2) on a microscope slide. Staphylococci colonies produce bubbles in H_2O_2 while streptococci do not.

Coagulase test (tube coagulase test) was done on cultures confirmed to be Staphylococci the Gram's staining and the catalase test. Three test tubes were prepared

and labelled; Test (T), Positive control (Pos) and Negative control (Neg). Rabbit plasma and peptone broth was prepared in the ratio 1:4. e.g. 0.2 ml of plasma to 0.8 ml of peptone. 0.2 ml of plasma was added to each of the tubes. 0.8 ml of sterile peptone was added to the Neg tube, and same volume of a suspension of the test organism added to the tube labeled T while 0.8 ml of control *S. aureus* suspension was added to the tube. The suspensions were homogeneously mixed and incubated at 37 °C for 18 to 24 h, and examined macroscopically for the presence of clot.

Statistical methodology

• The data from the study were entered into Microsoft excel spread sheet for data cleaning. The cleaned data was exported to SPSS (Statistical Package for the Social Sciences) version 23 software for analysis.

Description of qualitative variables was evaluated by frequency and percentage.

Description of quantitative variables was assessed in the form of mean and standard deviation (mean $\pm$ SD).

Chi-square test was used for comparison of qualitative variables with each other.

Comparison between quantitative variables was carried out using: Student t test, One way ANOVA test, Correlation and multivariable logistic regression analysis were performed. The significance of the results was assessed in the form of P- value that was differentiated into:

- Non-significant when P-value > 0.05.
- Significant when P-value ≤ 0.05
- Highly significant when P-value ≤ 0.001.
- The study was approved by Local Ethical Research Committee at Beni-Suef Faculty of Medicine: Approval No: FMBSUREC/08032020/ Abdel-Aziz**.**

3. Results:

The current study was conducted at the Neonatal Intensive Care Unit (NICU) of Beni-Suef University Hospital over a period of six months (April 2020 to September 2020). The population for the study comprised of 330 neonates admitted to NICU at Beni-Suef University Hospital in this period. A total of about average 24 Health care workers (HCWs) willingly accepted to participate in the study monthly.

Table (1): The characteristics of the studied neonates:

Variable	Measurement
Age (days) (Mean SD*; range (Min-max)	8.15 ±5.076 (1-21 day)
Gender N (%)	Male 110 (33.3)
	Female 220 (66.7)
Gestational age N (%)	Preterm 116 (35.2)
	Full term 214 (64.8)
Weight (gram) Mean SD (Min-max)	2707.12 ±612.74 (2000-4200)
Mode of Delivery N (%)	Normal 143 (43.3)
	C.S* 187 (56.7)

Table (1): illustrated that the mean age of the studied cases was 8.15 ±5.076 ranged from 1 day in minimum to 21 day maximum. About 110 (33.3) were males and 220 (35.2%) were females, out of the total studied cases 116 (35.2%) cases were preterm and 214 (64.8%) were full term. The weight at birth was 2707.12 ±612.74 ranged between (2000-4200). The mode of delivery was Normal in 143 (43.3%) of cases and Cesarean section in 187 (56.7) of cases.

Table (2): Distribution of nasal *S. aureus* carriage among healthcare

Occupation	N (%)	*S. aureus* carriers N (%)
Medicals doctors	15	0%
Nurses	9	0%
Total	24	0%

The table (2) showed that the total 24 HCWs included in the study were 15 doctors and 9 nurses and none of them was *S. aureus* carrier.

Table (3): Percent of *S. aureus* positive neonates on admission.

On Admission	Number	Percent
Negative	159	48.2
Positive	171	51.8
Total	330	100.0

This table (3) demonstrated that a number of 171 cases were positive at hospital admission.

Table (4): Frequency of S. aureus positive neonates on discharge.

On Discharge	Frequency	Percent
Negative	252	76.4
Positive	78	23.6
Total	330	100.0

This table (4) demonstrated that a number of 78 (23.6%) of all studied children considered were *S. aureus* carrier at hospital discharge.

Table (5): Comparison between different risk factors of studied neonates.

Variable	Unit	Positive No.=171	Negative No.= 159	P-value
Age (days)	Mean SD; range (Min-max)	10.64± 4.73 (1-21)	5.44±3.905	0.002*
Gender				
Male	N (%)	55 (32.2)	55 (34.6)	0.640
Female	N (%)	116 (67.8)	104 (65.4)	
Gestational age				
Preterm	N (%)	101 (59.1)	15 (9.4%)	
Full term	N (%)	70 (40.9)	144 (90.6)	0.001*
Weight (gram)	Mean SD (Min-max)	2657.54± 542.979	2760.45±677.544	0.128

Type of Delivery Normal C.S	N (%) N (%)	102 (59.6) 69 (40.4)	41(25.8%) 118(74.2)	0.001*

*C.S refers to Cesarean section, SD refers to standard deviation, Min refers to minimal, Max refers to maximal

Table (5) showed that older age is associated with positive culture results at screening and this was statistically significant p value 0.002. Also, preterm labor and normal delivery mode is associated with positive results and this was statistically significant p value 0.001.

Figure (1): Causes of admission to the studied neonates:

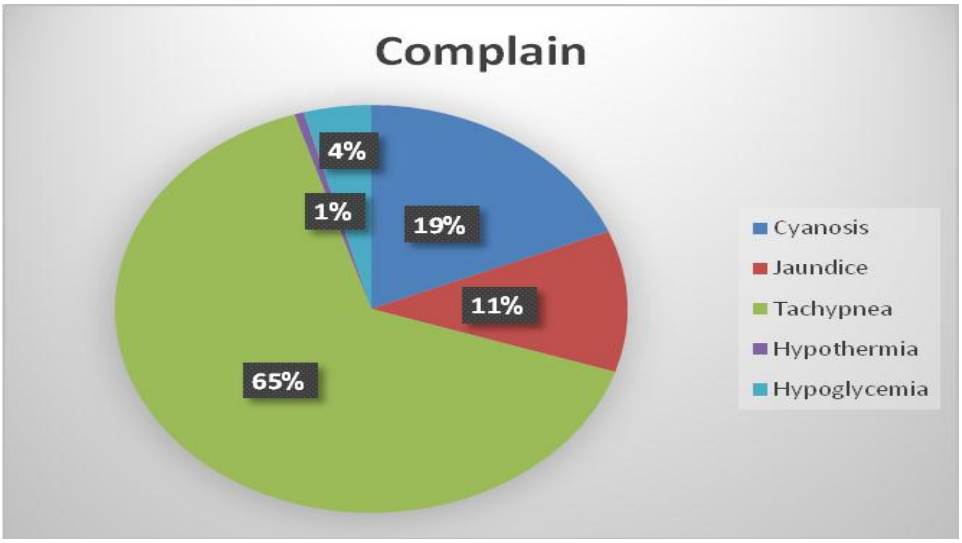


Table (6): Results of Blood cultures in neonates with *S. aureus* carriag

Blood culture	Screening Positivity on admission		P-value
	Negative	Positive	
S. aureus	3	54	0.001*
	(1.9%)	(31.6%)	
Non-Staph	156	117	
	(98.1%)	(68.4%)	
Total	159	171	
	100.0%	100.0%	

This table (6) demonstrated that those who are nasal staph carrier are associated with being staph positive in the blood culture and this association is statistically significant.

Figure (2): Results of blood culture during the studied period.

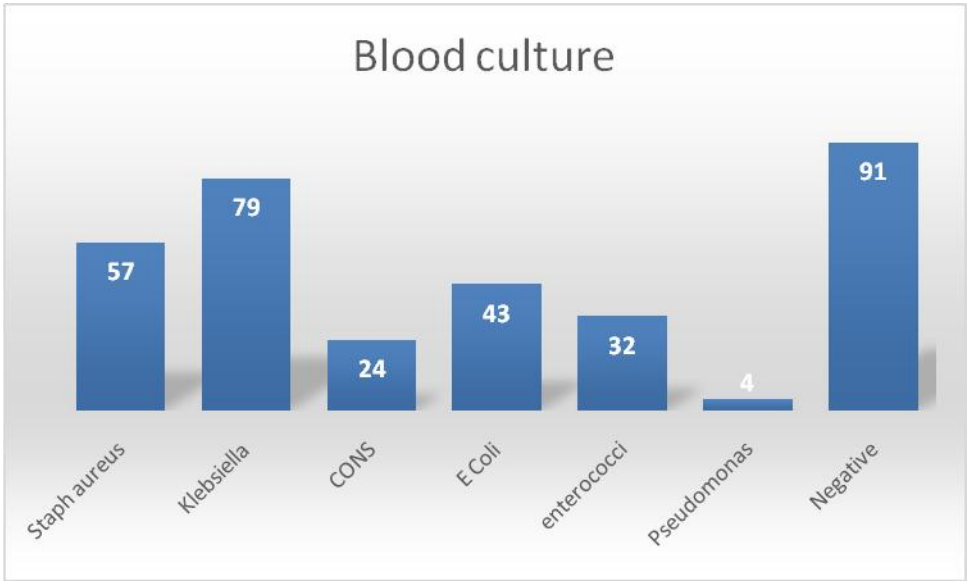
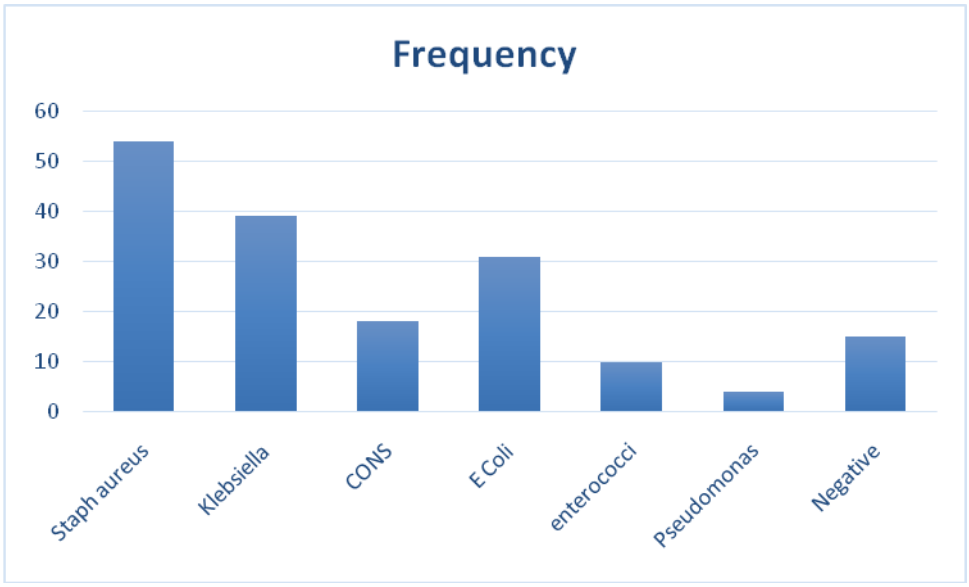


Figure (3): Results of Blood cultures in neonates within neonates with staph carriage



The following results analyze different risk factors for development of neonatal sepsis among the studied neonates

Table (7): frequency neonatal sepsis among the studied group:

Onset of sepsis		Frequency	Percent
Valid	less than 72 hour =early onset	171	51.8
	more than 72 hour= late onset	76	23.0
	No sepsis	83	25.2
	Total	330	100.0

Table (7) showed that 74% of studied neonates had sepsis (early onset 51.8% and late onset 23%) and 25.2% had no sepsis.

4. Discussion:

Hospital acquired infections (HAIs) occur in NICUs is a major health problem. These infections are one of the leading causes of mortality and morbidity in NICUs. In addition to the high costs, neonatal infections contribute to neurological disabilities and poor growth outcomes [8].

S. aureus is the second most common pathogen causing HAIs in neonates with an attributable high mortality rate. Despite aggressive measures to prevent *S. aureus* infections in neonates, the burden of *S. aureus* disease remains high in this population [9].Up to 40% of neonates acquire *S. aureus* in the first 2 months of life. Vertical transmission of *S. aureus* is rare, but postnatal transmission even to healthy infants is common in the first few months of life [10]

S. aureus associated healthcare-associated infections is an important health challenge. Sources of *S. aureus* infection in healthcare settings include colonized carriers or contaminated surfaces [11]

Neonatal intensive care unit (NICU) patients are at high risk of acquiring colonization and infection by *S. aureus* in NICUs was first reported in 1981; since then, the organism has been reported regularly in this age group and several outbreaks have been reported globally [12].The present study showed high prevalence of nasal *S. aureus* carriage 51%, this agrees with Arias et al., 2017 [13], who showed an increase in the prevalence of *S. aureus* carriage ranging from 7 to 53% between 1989 and 2017. Also, our finding agrees with van Hal et al., 2012 [14] who reported a high carriage rate of more than 30%. Additionally, in a previous study, performed between 2005 and 2006 in six Mexican hospitals, the prevalence of *S. aureus* ranged from 1 to 43% [15].

Zervou et al., 2014 [16] reported that neonatal nasal *S. aureus* carriage prevalence could range between 36% and 61%. The reasons for the high rates among neonates remain unclear. Possibly, colonization from parents, care givers or healthcare workers plays a critical role. Other possible reasons in our case could be cross contamination or infection from one baby to the other by healthcare workers transiently carrying the bacteria with contaminated hands. Several clinical factors may contribute to the *S. aureus* colonization. Previous antimicrobial use has been associated with the *S. aureus* colonization in hospitalized patients, particularly those in ICUs [17].

Other factors include the contact with a health-care facility (the length of stay, history of hospitalization) and personal medical background (diagnosis of skin and soft tissue infections, placement of nasal gastric tube) [18].

Our results showed that 5% (8/159) neonates in NICUs have acquired *S. aureus* during their hospital stay. Also [19].reported there was a relative risk of 24% for colonized patients in NICU to develop an *S. aureus* infection during hospitalization and he explained that due to an implication for infection control measures. Strict hand hygiene before and after patient contact is the primary measure in preventing and controlling the spread of *S. aureus*. Environmental hygiene is also important to reduce the *S. aureus* reservoirs and the transmission of *S. aureus* in the clinical setting [20].

In the current study by evaluating the complain on admission at NICU during the studied period, it was found that there was higher highly significant increase respiratory distress. This agreed with kresimir et al., 2017 [21] who stated that respiratory distress (RD) is a challenging problem and one of the most common causes of admission in NICU. Cochi et al., 2016 [22] also stated that respiratory distress was the most prevalent presentation of sepsis.

In the present study *S. aureus* carriage rate is absent among HCWs who participant in the study, possibly due to improved personal hygiene and well established immune system. This finding agreed with [23] who stated that no case was recorded in between the medical personnels. However, these results disagreed with EL-Shimi et al., 2014 [24] who found carrier rate among the sampled HCWs was 8.5% (9/106). Also we should know proper management of these patients and how to improve the quality of their life [25]. In the current study, *klebsiella* was detected in 23.9% of the results followed by *S. aureus* (17.3%). Our study found that nasal *S. aureus* colonization may be a predictor for subsequent invasive *S. aureus* infections. Neonates with nasal *S. aureus* colonization had significantly higher rates of subsequent *S. aureus* infections

than those without colonization (P = 0.001). This agreed with Anna et al., 2014 [26] who reported that nasal colonization may precede invasive infection with *S. aureus*.

The significant risk factors associated with neonatal sepsis in our study were UTI (63.6 %), premature rupture of membrane (PROM) > 18 hours (17.4%) hypertension (7.3%) and obstructed labor (11.7%). Maternal risk factors were studied by (Abd El Haleim et al., 2019) [27] in Cairo University where eclampsia in (16.7%) of patients and premature rupture of membranes (PROM) > 12 hours was reported in (83.3%)

5. Conclusion and Recommendations:

Hospital associated infections is one of the leading causes of morbidity and mortality in neonates in NICU and affect the duration of their hospitalization, as well as the quality of their care. The primary purpose of infection carriage surveillance is to prevent and monitor healthcare associated infections with subsequent significant decrease in incidence of outbreaks or multidrug-resistant organisms. Continuous assessment and screening of nasal *S. aureus* carriage is beneficial for decreasing neonatal sepsis**.**

6. References:

1. Blencowe H, Cousens S, Oestergaard M, Chou D, Moller AB, Narwal R, Adler A, Garcia CV, Rohde S, Say L, and Lawn JE. National, regional and worldwide estimates of preterm birth. The Lancet. 2012; 379(9832):2162-72
2. Matary A, Heena H, AlSarheed AS, Ouda W, AlShahrani DA, Wani TA, et al. (2019): Characteristics of neonatal Sepsis at a tertiary care hospital in Saudi Arabia. J Infect Publ Health 2019; 12(5): 666e672.
3. Quddus AR, Islam MN, Uddin MB, Mahmud AA, Badruzzaman M, Saha SK, et al. (2019): Study of Risk Factors, Causative Organisms & Their Sensitivity Pattern in Neonatal Sepsis in a Community Based Tertiary Level Hospital. Mymensingh Med J [Internet]. 2019 Oct [cited 2020 Apr 8]; 28(4):839–48.
4. Garba B, Muhammad A, Mohammed B, Obasi A, Adeniji A. (2017): A study of neonatal mortality in a specialist hospital in Gusau, Zamfara, North-Western Nigeria. Int J Trop Dis Heal. 2017;28:1–6.
5. Edwards MS, Baker CJ. (2014): Sepsis in the newborn, p 545–561. In Gershon AA, Hotez PJ, Katz SL (ed), Krugman's infectious diseases of children, 11th ed. Mosby, Philadelphia, PA.
6. Kluytmans J, van Belkum A, Verbrugh H. (2019): Nasal carriage of *S. aureus*: epidemiology, underlying mechanisms and associated risks. Clin Microbiol Rev. July 2019;10 (3):505–20.
7. Paul MO, Lamikanra A, Aderibigbe DA. (2019): Nasal carriers of coagulase positive staphylococci in a Nigerian hospital

community. Trans. R. Soc. Trop. Med. Hyg. 2019;76 (3):319–23.

8. Popoola VO, Budd A, Wittig SM, et al. (2014): *S. aureus* Transmission and Infections in a Neonatal Intensive Care Unit despite Active Surveillance Cultures and Decolonization: Challenges for Infection Prevention. Infect Control Hosp Epidemiol. 2014;35(04): 412–418.
9. Leshem E, Maayan-Metzger A, Rahav G, et al. (2012): Transmission of *S. aureus* from mothers to newborns. Pediatr Infect Dis J 2012;31:360–3.
10. Jimenez-Truque N, Tedeschi S, Saye EJ, et al. (2012): Relationship between maternal and neonatal Staphylococcus aureus colonization. Pediatrics 2012; 129: e1252–9.
11. Iacobelli S, Colomb B, Bonsante F, Astruc K, Ferdynus C, Bouthet MF, et al. (2013): Successful control of a S. aureus outbreak in a neonatal intensive care unit: a retrospective, before-after study. BMC Infect Dis. 2013;13(1):440.
12. World Health Organization. (2016): Five moments for hand hygiene. Geneva: World Health Organization; 2016 (http:// www.who.int/gpsc/ tools/Five_ moments/en/).
13. Arias CA, Reyes J, Carvajal LP, Rincon S, Diaz L, Panesso D, et al. (2017): A prospective cohort multicenter study of molecular epidemiology and phylogenomics of *S. aureus* bacteremia in nine Latin American countries. Antimicrob Agents Chemother. 2017;61(10).
14. van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB.(2012): Predictors of mortality in *S. aureus* bacteremia.Clin Microbiol Rev. 2012;25(2):362–86.
15. Martínez Aguilar G. Análisis de genotipos y de los tiempos (2010): duplicación de cepas de *S. aureus* resistente de infecciones nosocomiales y adquiridas en la comunidad. Diss. PhD thesis. México: Universidad de Colima; 2010.
16. Zervou FN, Zacharioudakis IM, Ziakas PD, Mylonakis E. (2012): *S. aureus* colonization and risk of infection in the neonatal and pediatric ICU: a meta-analysis. Pediatrics. 2014;133(4):e1015–23.
17. Gesualdo F, Bongiorno D, Rizzo C, Bella A, Menichella D, Stefani S, et al. (2013): SA nasal colonization in children: prevalence meta-analysis, review of risk factors and molecular genetics. Pediatr Infect Dis J. 2013;32 (5):479–85.
18. Marimuthu K, Pittet D, Harbarth S. (2014): The effect of improved hand hygiene on nosocomial MRSA control. Antimicrob Resist Infect Control. 2014;3:34.
19. Dramowski, A. Madide, and A. Bekker, (2015): "Neonatal nosocomial bloodstream infections at a referral hospital in a middle-income country: burden, pathogens, antimicrobial resistance and mortality," Paediatrics and International

Child Health, vol. 35, no. 3, pp. 265–272, 2015.

20. Pierce R, Lessler J, Popoola VO, Milstone AM. (2017): *S. aureus* acquisition risk in an endemic neonatal intensive care unit with an active surveillance culture and decolonization programme. J Hosp Infect. 2017; 95(1):91–97.
21. Kresimir M, Hana D, Matej S, Krunoslav M, Katarina P, Vvesna M, Karolina K (2017): Causes of respiratory distress among neonates of gestational age 32 weeks and more. Journal in Intensive Care And Emergency Medicine, 13(4): 21 – 24.
22. Cochi SE, Kempker JA, Annangi S, et al (2016): Mortality trends of acute respiratory distress syndrome in the United States from 1999–2013. Ann Am Thorac Soc., 13: 1742–1751.
23. Mike S., JOLANTA W. and ANNA S. (2014): Serum and Urinary examination in Newborns, Bio. Med. Research International, (24): 1-8, 2014.
24. EL-Shimi M.S., MOHAMED M.H., ABDEL AL AHMED, et al. (2014): Human Neutrophil Lipocalin in Early Diagnosis of Neonatal diseass. Egyptian Journal of Pediatrics, 394 (3587): 1-15, 2014.
25. Nabila Ibrahim Abd El-Megeed Laza , Mohamed Nabil Salemb , Hebatullah Kamal Ebraheem Tahaa , Mona Mahmoud Sroura (2020): Studying the characteristics of interstitial pneumonia with auto immune features Chest diseases and tuberculosis, Faculty of Medicine, Beni Suef University, Egypt. Internal medicine. Rheumatology & Immunology, Faculty of Medicine, Beni Suef University, Egypt. Egyptian Journal of Medical Research (EJMR), Volume 1, Issue2, 2020.
26. Anna CS, Hannah B, Alexander AM, et al. (2014): Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, South Asia, and Latin America for 2012: a systematic review and meta-analysis. Lancet. 2014; 14: 731 –40.
27. Abd El Haleim M. Mona, Nada N. Nawar, Eman M. Abd El Rahman, Heba H. Abo Hussein and Nermin R. Mohamed Kamel (2019): Epidemiologic and Micro-bacteriologic Study of Neonatal Septicaemia in Cairo University Neonatal Intensive Care Units Clinical Pathology Department, Faculty of Medicine, Cairo University, Egypt Research Journal of Medicine and Medical Sciences: no. 18, pp. 1093–1147, 2019.