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Case Report **Open Access**

Isolation of *Globicatella sanguinis* from a neonate with sepsis using BacT/Alert and VITEK-2 compact system at Federal Teaching Hospital, Katsina, northwest Nigeria: A case report

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Abstract:

Neonatal sepsis is a significant cause of neonatal morbidity and mortality, predominantly in developing countries. The bacterial causes of neonatal sepsis and their antimicrobial susceptibility patterns are however dynamic. *Globicatella sanguinis* is a streptococcus-like bacterial agent capable of causing serious infection in humans that has been rarely isolated from clinical samples, and is an uncommon pathogen that is difficult to identify. Identification based on phenotypic methods alone can misidentify many bacteria, and this may affect precise antibiotic treatment. We report the isolation of a rare bacterial pathogen, *G. sanguinis* from a three-hour-old preterm female neonate (28 weeker, extremely low birth weight) with sepsis and respiratory distress syndrome (RDS) at Federal Teaching Hospital Katsina, Nigeria, and to the best of our knowledge, one of the very few reported cases all over the world. Blood sample was aseptically collected from the neonate and cultured on BacT/Alert automated system (BioMérieux, Mercy-Etoile, France). A rare bacterium was identified from a positive culture, and *in vitro* susceptibility test using VITEK-2 compact system showed the isolate to be sensitive to gentamicin, cefuroxime, ceftriaxone, and ceftazidime. Despite antibiotic treatment and other standards of care, on day 9 of admission, the baby developed apnea and all resuscitative measures proved abortive. In a developing country like Nigeria where child mortality due to infection is high, the inclusion of advanced technologies such as improved VITEK-2 compact system, PCR, MALDI-TOF MS, and next-generation sequencing, could play a significant role in its reduction.

Keywords: *Globicatella sanguinis*; neonate; sepsis; respiratory distress syndrome

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Isolement de *Globicatella sanguinis* d'un nouveau-né atteint de septicémie à l'aide du système compact BacT/Alert et VITEK-2 au Federal Teaching Hospital de Katsina, au nord-ouest du Nigéria: à propos d'un cas

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Résumé :

La septicémie néonatale est une cause importante de morbidité et de mortalité néonatales, principalement dans les pays en développement. Les causes bactériennes du sepsis néonatal et leurs profils de sensibilité aux antimicrobiens sont cependant dynamiques. *Globicatella sanguinis* est un agent bactérien de type streptocoque capable de provoquer une infection grave chez l'homme qui a rarement été isolé à partir d'échantillons cliniques, et est un agent pathogène

rare et difficile à identifier. L'identification basée sur des méthodes phénotypiques seules peut mal identifier de nombreuses bactéries, ce qui peut affecter un traitement antibiotique précis. Nous rapportons l'isolement d'un agent pathogène bactérien rare, *G. sanguinis* chez un nouveau-né prématuré de trois heures (28 semaines, poids de naissance extrêmement faible) atteint de septicémie et de syndrome de détresse respiratoire (SDR) à l'hôpital fédéral d'enseignement de Katsina, au Nigéria, et à notre connaissance, l'un des très rares cas signalés dans le monde. L'échantillon de sang a été prélevé de manière aseptique sur le nouveau-né et cultivé sur le système automatisé BacT/Alert (BioMérieux, Mercy-Etoile, France). Une bactérie rare a été identifiée à partir d'une culture positive, et un test de sensibilité *in vitro* utilisant le système compact VITEK-2 a montré que l'isolat était sensible à la gentamicine, au céfuroxime, à la ceftriaxone et à la ceftazidime. Malgré un traitement antibiotique et d'autres normes de soins, au jour 9 de l'admission, le bébé a développé une apnée et toutes les mesures de réanimation se sont avérées avortées. Dans un pays en développement comme le Nigeria où la mortalité infantile due à l'infection est élevée, l'inclusion de technologies de pointe telles que le système compact VITEK-2 amélioré, la PCR, la SM MALDI-TOF et le séquençage de nouvelle génération pourrait jouer un rôle important dans sa réduction.

Mots-clés: *Globicatella sanguinis*; nouveau-né; état septique; syndrome de détresse respiratoire

Introduction:

Neonatal sepsis is a substantial cause of neonatal morbidity and mortality, particularly in developing countries. The microbial agents implicated in neonatal sepsis and their antimicrobial susceptibility patterns are however dynamic (1). Neonatal sepsis is a clinical condition comprising of non-specific symptoms and signs of infection, accompanied by bacteremia in the first 28 days of life. It is a systemic infection occurring in the neonatal period (2). Bacterial infection is mostly implicated in about three-quarters of the million neonatal mortality each year, all over the world. (3), this is because the neonatal period is the most vulnerable period of life due to susceptibility to infectious agents, as neonates are deficient in both humoral and cell-mediated immunity. They also produce immunoglobulins at a much lower proportion when compared to older children and adults (4).

Neonatal sepsis can be categorized as early-onset sepsis (between 0 to 3 days of life) or late-onset sepsis (from day 4 or later). Early-onset sepsis is commonly connected with the acquisition of microbes from the mother, and onset is mostly immediate in premature babies. Infection can arise through hematogenous, transplacental spread from an infected mother or more commonly, through ascending infection from the vaginal. The baby may acquire organisms that colonize the genitourinary tract of the mother as it passes through the birth canal during delivery. The common bacterial causes of early-onset sepsis include group B streptococcus (GBS), *Escherichia coli*, coagulase negative staphylococcus, *Haemophilus influenzae*, and *Listeria monocytogenes* (5). Early-onset sepsis is about 10 to 20 times more likely in premature and very low birth weight babies (6).

Late-onset sepsis occurs after 4 days of life and it is characteristically acquired from the environment. Bacteria that have been implicated include coagulase-negative staphylococcus, *Staphylococcus aureus, Escherichia coli, Klebsiella, Pseudomonas, Enterobacter,* group B streptococcus, *Serratia*, *Acinetobacter,* and anaerobes. The skin, respiratory tract, conjunctivae, gastrointestinal tract, and umbilicus of neonate might become colonized through interactions with the environment or caregivers (5).

Empirical antibiotic treatment initiated to lessen the deadly outcomes of sepsis in neonates is based on the knowledge of common pathogens encountered in a given locality, as well as their sensitivity patterns (3). In developed countries, the most common organisms causing neonatal sepsis are Group B streptococci, *E. coli,* and *L. monocytogenes* while Gram-negative bacteria and coagulase-negative staphylococci are the most implicated in developing countries (7). But in recent years the emergence of rare bacterial pathogens is being witnessed (8,9) which may or may not be covered by empirical antibiotic treatment.

The 'gold standard' for the diagnosis of sepsis in neonates is a positive culture from a disinfected site, including blood, cerebrospinal fluid (CSF), or urine (10). Here we report the isolation of a rare bacterial pathogen, *Globicatella sanguinis,* a streptococcus-like agent from a three-hour-old preterm neonate with sepsis, and to the best of our knowledge one of the few reported cases so far, in Nigeria.

Case report:

A 3-hour-old female preterm neonate was referred to Federal Teaching Hospital (FTH), Katsina, on account of prematurity, difficulty in breathing, and inability to feed. The neonate was born at home to a 20-year-old primigravida by spontaneous vaginal delivery at about 28 weeks of gestation, with a birth weight of 750 grams. Pregnancy was unbooked and the duration of labor was about seven hours with spontaneous premature rupture of membrane (PROM) for about half an hour prior to labor.

On examination, the neonate was pink in room air, lethargic, afebrile, anicteric, acya-

nosed, not dehydrated, and had no pedal edema. Respiratory rate was 48cpm with oxygen saturation (SPO_2) of 67% on continuous positive airway pressure (CPAP). Temperature was 33.2^{o}C (hypothermia) and PCV was 48%. An assessment of preterm extremely-low-birth-weight (ELBW) 28-weeker with suspected sepsis was made based on clinical features of lethargy, inability to feed, respiratory distress, and hypothermia.

Approximately 2 milliliters of blood were aseptically collected from the peripheral vein of the neonate into an appropriately labeled Bact/Alert paediatric bottle, which was then moved into a closed flask and instantly transported to the laboratory for analysis. Baby was placed on empirical antimicrobial combinations of gentamicin and cefuroxime, as well as other supportive management, in line with the hospital neonatal unit protocol, pending the outcome of the blood culture.

Bacterial detection with BacT/Alert blood culture system:

Inoculated bottles were placed in BacT/Alert microbial detection system, which utilizes a colorimetric sensor and reflected light to detect the presence of carbon dioxide (CO_2) produced when bacteria (if present in the blood sample) grow and metabolize the substrates in the culture medium, with the color of the gas-permeable sensor installed in the bottom of each culture bottle changing to yellow (11).

The bottles were incubated aerobically and continuously observed for the presence of microbial growth for 5 days. Culture-positive bottle was removed for bacterial identification by Gram stain smears and sub-cultures on Blood and MacConkey agar plates. The procedures for loading and unloading the culture bottles into the BacT/Alert machine were done in line with the manufacturers' user-instruction manual.

Bacterial identification and antibiotic susceptibility test using VITEK-2 compact system:

The bacteria inoculum was prepared from pure colonies of the isolates on the agar plate and the turbidity adjusted to 0.5 McFarland standard using the Vitek-2 Densi-Check instrument. Bacterial identification with Vitek-2 compact system was performed according to manufacturer's instructions using the 64-well card. The computerized Vitek-2 compact system (30-card capacity) uses a fluorogenic technique for microbial identification and a turbidimetric technique for susceptibility testing to generate minimum inhibitory concentration (MIC) data with a 64-well card, barcoded with data on card type, expiration date, lot number, and unique card identification number (12,13).

The bacterial culture suspension was inoculated into the appropriate card using an integrated vacuum device inside the filling chamber within 30 minutes of inoculum preparation. A test tube holding the suspension was placed in a distinct rack (cassette) and the identification card was placed in the adjacent slot while implanting the transfer tube into the corresponding suspension tube. The cards were later transferred into the loading chamber where they were sealed and incubated in a rotating carousel at 37^{o}C. Upon loading the cassette, the machine handles all successive steps for inoculation and interpretation with regards to identification and susceptibility test, and the AST results were available for bacteria isolate in less than 18 hours. Eight pre-installed antibiotics (gentamicin, ciprofloxacin, cefuroxime, ceftriaxone, ceftazidime, augmentin, meropenem, and vancomycin) were tested against the isolate.

Globicatella sanguinis was identified by the system, sensitive to gentamicin, cefuroxime, ceftriaxone and ceftazidime. Despite antibiotic treatment and other standards of care given, on day 9 of admission, the baby developed apnea and all resuscitative measures proved abortive. For religious and cultural reasons, autopsy was not performed.

Discussion:

Globicatella sanguinis is an uncommon pathogenic Gram-positive coccus that has been occasionally reported as an unusual cause of infections of the bloodstream, central nervous system, endocardium, and urinary tract in man. The other member of the genus, *G. sulfidifaciens*, is not a known pathogen in humans (9). The clinical and epidemiological implications of *G. sanguinis* remain to be determined, even though published data suggest its opportunistic nature and the ability to cause serious infections. (8). The case in this study is one of the very few reported cases of isolation of *G. sanguinis* from a neonate in Nigeria and all over the world (8,14,15,16). Its clinical implication in humans was documented on some rare occasions where it was isolated from urine, blood, and cerebrospinal fluid in cases of urinary tract infections, bacteremia, and meningitis respectively (17,18).

Globicatella sanguinis is a facultatively anaerobic, alpha-haemolytic, and catalase negative bacterium. Its identification and characterization with conventional laboratory tests are problematic, as there exists various descriptions of biochemical reactions for the same species (19). The colony morphology of the strains

makes it difficult to distinguish from aerococci, viridans streptococci, and enterococci. Although ability to grow in 6.5% NaCl and failure to grow at 10°C helps to differentiate *Globicatella* from viridans streptococci and enterococci respectively, it is hard to distinguish it from *Aerococcus* (20). Standardized systems such as Rapid ID 32 Strep or API 20 Strep were recognized to identify alpha-hemolytic streptococci (21) but these are not readily available in our setting.

In view of the growing emergence of multi-drug-resistant bacteria, the rational use of antibiotics based on accurate and reliable laboratory results becomes an absolute necessity. This can only be achievable through accurate identification of pathogens as well as treatment based on sensitivity patterns. There is limited knowledge on antibiotic susceptibility patterns as far as this rare bacterium is concerned, all over the world, however, the *in-vitro* antibiotic susceptibility based on VITEK-2 compact system in this case report shows that the bacterium is sensitive to gentamicin, cefuroxime, ceftriaxone and ceftazidime. The neonate was placed on empirical combination of gentamicin and cefuroxime, as well as other supportive management, and may have died from complications of RDS. RDS is significantly associated with poor outcomes of neonatal sepsis (22) .

Although more evaluation of the presence and nature of *G. sanguinis* in uninfected persons is needed, this bacterium may be part of the lower gastrointestinal or urogenital microbiome of the mother, with the potential to cause disease in vulnerable hosts. Its low prevalence may be partly due to the difficulty in laboratory identification and characterization by phenotypic methods, hence the recognition of this pathogen will be enhanced as advanced technologies such as improved VITEK-2 compact system, MALDI-TOF MS, and next-generation sequencing techniques emerge into common use.

Conclusion:

In this report, we highlight the fact that identification solely based on phenotypic methods can misidentify many bacteria, and this may affect precise antibiotic treatment. Due to problems with identification and characterization, and the fact that there is a small number of reported cases, the pathogenic significance of *G. sanguinis* is still only partly known. The bacterium is hardly encountered in medical laboratories, hence technicians, microbiologists as well as clinicians are not adequately familiar with the phenotypic characteristics and identification peculiarities. To this end, the pathogen may be unnoticed when isolated or simply reported as unidentified streptococcus-like isolate.

In developing countries where child mortality due to infection is high, inclusion of advanced technologies such as improved VITEK-2 compact system, PCR, MALDI-TOF MS, and next -generation sequencing, could play a significant role in its reduction.

Contributions of authors:

OHK, SMB, YSA, SA, and ABT conceived and developed the idea of the case report. OHK performed the laboratory work. All authors discussed the results and contributed to the final manuscript.

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Conflict of interest:

Authors declared no conflicts of interest.

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