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Emergence of *Raoultella ornithinolytica* as a significant intestinal microbiota in Nigerian Neonates.

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Abstract

Background: There are increasing reports of *Raoultella ornithinolytica* infection in humans. This study reports significant contribution of *Raoultella ornithinolytica* to neonatal gut microbiota.

Methods: Rectal swab samples were collected in 70 healthy neonates, within 6-12hours of birth, and days 3, 9, and 14 after birth. Colonization by *R. ornithinolytica* was evaluated against neonatal characteristics.

Results: Among the 70 neonates, *R. ornithinolytica* was the fourth most common bacteria isolated. *R. ornithinolytica* was part of gut microbiota at birth, and on Day 3, Day 9 and Day 14 after birth in 15%, 28.6%, 21.4% and 5.7% of neonates respectively. No factor was associated with *R. ornithinolytica* colonization, but a trend towards an increased likelihood of colonization among females, asphyxiated and neonates whose mothers had prolonged labour was observed.

Conclusion: R. ornithinolytica has emerged as an important part of gut microbiota among neonates in Nigeria, but its' role in neonatal dysbiosis and infection remains unclear.

Keywords: Gut microbiota, infection, Neonate, *Raoultella ornithinolytica*

Abstrait

Contexte : De plus en plus de cas d'infection à *Raoultella ornithinolytica* sont rapportés chez l'homme. Cette étude rapporte une contribution significative de *Raoultella ornithinolytica* au microbiote intestinal néonatal.

Méthodes : Des échantillons d'écouvillons rectaux ont été prélevés sur 70 nouveau-nés en bonne santé, dans les 6 à 12 heures suivant la naissance et les jours 3, 9 et 14 après la naissance. La colonisation par *R. ornithinolytica* a été évaluée par rapport aux caractéristiques néonatales.

Résultats : Parmi les 70 nouveau-nés, *R. ornithinolytica* était la quatrième bactérie la plus couramment isolée. *R. ornithinolytica* faisait partie du microbiote intestinal à la naissance et aux jours 3, 9 et 14 après la naissance chez 15 %, 28,6 %, 21,4 % et 5,7 % des nouveau-nés respectivement. Aucun facteur n'a été associé à la colonisation par *R. ornithinolytica*, mais une tendance à une probabilité accrue de colonisation chez les femelles, les asphyxiées et les nouveau-nés dont les mères ont eu un travail prolongé a été observée.

Conclusion : R. ornithinolytica est apparu comme une partie importante du microbiote intestinal chez les nouveau-nés au Nigeria, mais son rôle dans la dysbiose et l'infection néonatales reste incertain.

Mots-clés : *Microbiote intestinal, infection, Nouveau-né, Raoultella ornithinolytica*

Introduction

Gut microbiota plays important roles in the digestive, metabolic, immune and neurological functions and consequently helps to understand dysbiosis and diseases [1]. Neonatal microbiota is also an important determinant of aetiologic agents in neonatal sepsis. More recently, attention has been drawn to the role of emerging intestinal microbiota in facilitating bacterial translocation to other tissues in infants [2, 3]. Understanding this emerging intestinal microbiota may explain some of the challenges faced in the management of neonatal septicaemia in settings with resource-limited laboratory diagnostic facilities.

Despite advances in diagnosis and treatment, bacterial sepsis remains a significant cause of morbidity and mortality among neonates in developing countries [4]. Bloodstream infection may arise through bacterial translocation across the airways, gastrointestinal tract, genital tract, or breaks in the skin during insertion of medical devices [5]. Bacterial sepsis among neonates is often a consequence of this bacterial infection or microbial products released into the bloodstream, which results in systemic inflammatory response syndrome [6, 7].

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Common aetiologic agents in neonatal sepsis in developing countries have not reported aetiologic agents similar to those from developed countries [8, 9], however, reports from developing countries do not routinely screen for uncommon bacteria like *Raoultella ornithinolyitca*.

Over the past decade, *R. ornithinolyitca* has emerged as an infrequent, but important causal agent of human infections and has been documented to cause fatal infections in neonates [10-12]. *R. ornithinolyitca* is a gram negative, non-motile, encapsulated, aerobic bacillus previously named *Klebsiella ornithinolytica* [13]. *R. ornithinolyitca* belongs to the family *Enterobacterales* and has been isolated from insects, fish and brackish water. This bacterium, along with the closely related species *Raoultella plan-ticola*, has been shown to be the causative agent of histamine toxicity from fish (also known as scombroid syndrome) [14], but it is frequently misidentified as *Klebsiella pneumonia* [14]. The role of *R. ornithinolyitca* in neonatal infections remains unclear, but few studies have alluded to its' emerging role in neonatal intestinal colonization [15] and infections [12]. Podschun *et. al*. reported a prevalence of 8.7 % of *Raoultella spp.* among *Klebsiella spp*. colonising neonates at admission in neonatal wards in Germany [16].

Evolution of neonatal microbiota is affected by various factors such as the age at delivery, mode of delivery and antibiotic exposure [17, 18]. There is a relative lack of information about possible factors that may influence the ability of *R. ornithinolytica* to colonize the intestine in the neonatal period and consequent infections. Important sources of the microbiota colonization of the mucosal surfaces, the gut and the skin of the new born include vertical transmission of maternal microbiota and vaginal delivery with colonization of the gut beginning probably before and at the time of birth [19, 20].
This report, a part of an earlier publication [21], describes the intestinal colonization in the first two weeks of life by *R. ornithinolytica* among Nigerian neonates and associated factors.

Methods

As reported earlier by Kigbu *et al* [21], this longitudinal study involved 70 apparently healthy neonates. The neonates were recruited at the University College Hospital (UCH), Ibadan within 6–12 h of birth, and subsequently followed up on days 3, 9, and 14 after birth. Details of study methods have been previously published [21].

In brief, information on mothers' demographic characteristics, pregnancy and delivery history; neonates' characteristics including APGAR scores, mode of resuscitation, feeding methods, and antibiotic/antimycotic use were collected. Rectal swab samples were collected at birth and on days 3, 9, and 14 after birth. Standard microbiological methods were used to process the rectal samples and pathogen identification of all the gram-negative isolates were done using the Analytical Profile Index (API) 20E® and API 20NE® (API® bioMérieux Clinical Diagnostics Kits, USA) identification system. Gram-positive cocci were identified biochemically using the catalase test and coagulase test; Pastorex for *Staphylococci spp,* while anaerobes were identified with AnaeroGen, a miniaturized GasPak™ gas regenerating kit system.

Data analysis

The main outcome was proportions of *R. ornithinolytica* among isolates cultured on days 1, 3, 9, and 14 while independent variables included characteristics of neonates and mothers. Chi-square test was used to test associations between categorical variables, while comparisons between continuous variables were performed using Student's *t*-test. The patterns of organisms identified on the different days were displayed in charts. All $p < 0.05$ were considered statistically significant. The data were analysed using Statistical Package for Social Sciences (SPSS) for Windows 21 (IBM SPSS statistics 2012, Chicago, IL, USA).

Ethical considerations

Approval for the study was obtained from the University of Ibadan/University College Hospital, Ibadan Ethics Review Committee (approval number: UI/ EC/13/0161), and written informed consent was obtained from each mother.

Results

This study involved a secondary analysis of a cohort of neonates. The neonates' general characteristics have been published earlier by Kigbu *et al* [21]. In the present analysis, of the 70 neonates who had rectal samples collected, *R. ornithinolytica* was the fourth most common bacteria isolated, after coagulase negative S*taphylococcus*, *staphylococcus aureus* and *Escherichia coli*. This pattern remained consistent through the first 14 days of life (Fig 1). In all, 15% of the neonates were colonized by *R. ornithinolytica* at birth, this peaked at 28.6% on day 3 and dropped off to 5.7% on day 14 of life (Fig 2).

There was a trend towards increased likelihood of being colonized by *R. ornithinolytica* among female neonates and those with asphyxia or neonates whose mothers had prolonged labour (Table 1).

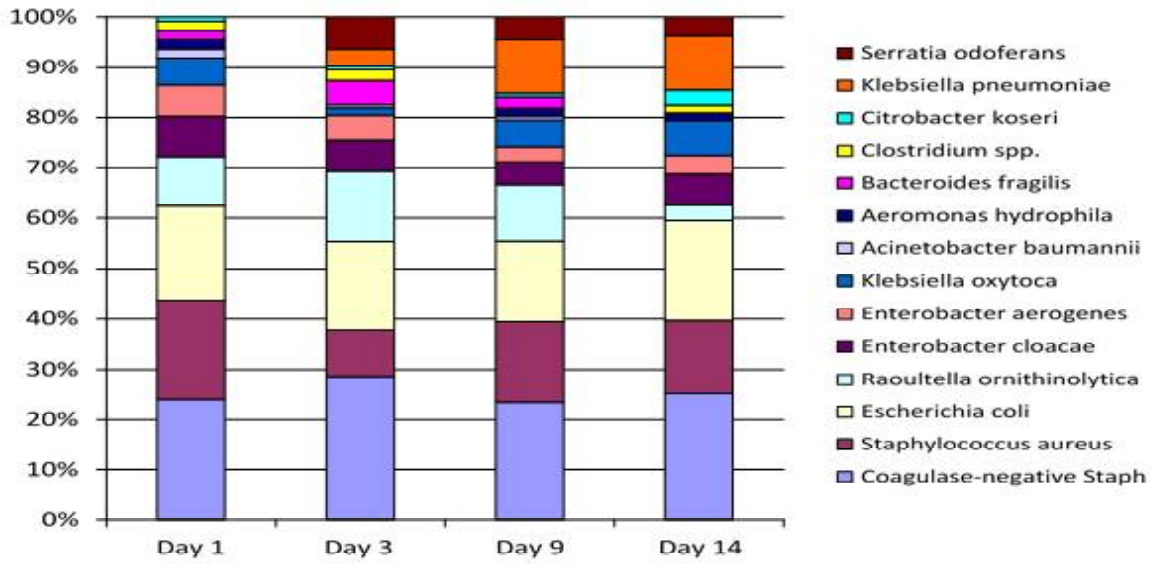

Fig 1: Pattern of bacterial colonization among the neonates

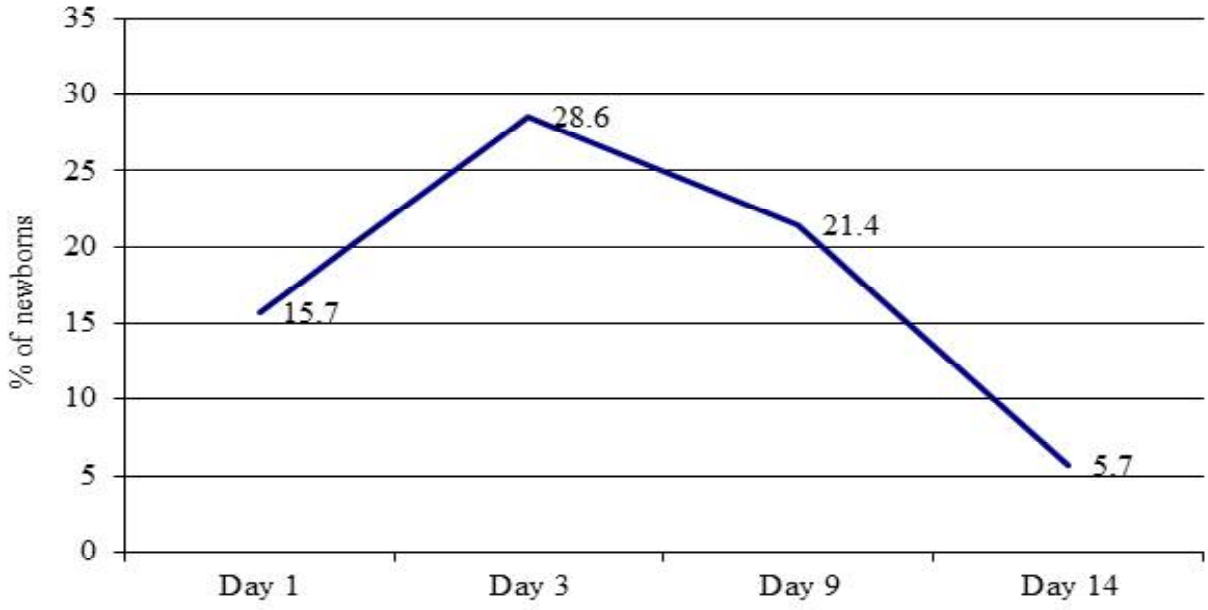

Fig 2: Trend of *R. ornithinolytica* colonization in the first 14 days of life among the neonates

Discussion

There are limited studies that evaluated the intestinal microbiota in African neonates [22]. The diversity of bacteria in the stools of new born infants have been demonstrated [22], though *R. ornithinolytica* has been rarely reported [10, 23]. The pattern of the bacterial pathogens responsible for neonatal sepsis has changed with time, geographical location and between the developed and developing countries [22, 24]. This study identified *R. ornithinolytica* in 15 % of colonized neonates at birth and the proportion remained significant through the first 2 weeks of life. However, a trend of decreasing prevalence over the

Table 1: Association between *R. ornithinolytica* colonization and neonatal risk factors

	Day 1			Day 3			Day 9			Day 14		
	Yes	No	OR (95% CI)	Yes	No	OR (95% CI)	Yes	No	OR (95% CI)	Yes	No	OR (95% CI)
Gender												
Female	8 (21.1)	30 (78.9)	2.58 (0.62, 10.68)	10(26.3)	28(73.7)	0.78(0.28, 2.22)	8(21.1)	30(78.9)	0.95 (0.30, 3.0)	0(0.0)	38(100.0)	1.14(1.0, 1.3)
Male	3 (9.4)	29 (90.6)		10(31.2)	22(68.8)		7(21.9)	25(78.1)		4(12.5)	28(87.5)	
Maturity												
Preterm	1 (5.6)	17 (94.4)	0.25 (0.03, 2.08)	5(27.8)	13(72.2)	0.95(0.29, 3.13)	2 (11.1)	16 (88.9)	0.38 (0.08, 1.85)	2 (11.1)	16 (88.9)	3.13(0.41, 24.01)
Term	10 (19.2)	42 (80.8)		15(28.8)	37(71.2)		13 (25.0)	39 (75.0)		2 (3.8)	50 (96.2)	
Mode of delivery												
SVD	10 (15.4)	55 (84.6)	0.73 (0.07, 7.20)	18 (28.6)	45(71.4)	1.60(0.17, 15.31)	14 (22.2)	49(77.8)	1.14 (0.12, 11.07)	3 (4.8)	60 (95.2)	0.20(0.02, 2.39)
CS	1 (20.0)	4 (80.0)		1(20.0)	4(80.0)		1(20.0)	4(80.0)		1 (20.0)	4(80.0)	
Presence of Asphyxia												
Yes	1 (9.1)	10 (90.9)	0.49 (0.06, 4.27))	2(18.2)	9(81.8)	0.51 (0.10, 2.58)	0(0.0)	11 (100.0)	1.34 (1.16, 1.56)	0 (0.0)	11 (100.0)	1.07(1.0, 1.15)
No	10 (16.9)	49 (83.1)		18 (30.5)	41 (69.5)		15 (25.4)	44 (74.6)		4 (6.8)	55 (93.2)	
Prolonged labor												
Yes	3 (25.0)	9 (75.0)	2.08 (0.46, 9.38)	4(33.3)	8(66.7)	1.27(0.33, 4.84)	1(8.3)	11 (91.7)	0.28 (0.03, 2.38)	0 (0.0)	12 (100.0)	1.06(0.99, 1.13)
No	8 (13.8)	50 (86.2)		15(28.3)	38(71.7)		13 (24.5)	40 (75.5)		3 (5.7)	50 (94.3)	

*SVD-Spontaneous vaginal delivery, CS- cesarean section

two-week period of study was observed. This is the

first report of *R. ornithinolytica* as a part of neonatal intestinal microbiota in Africa.

Using metagenomic sequencing on stools of neonates in Gabon and the Central African Republic, bacterial diversity is reported to be high from the first day of life. Brazier *et al* documented predominance of the genus *Prevotella spp in* meconium, which rapidly decreased through day 28 of life when the gut flora was composed almost exclusively of anaerobic bacteria [22].

Furthermore, Brazier *et al* [22] demonstrated that the infant's faecal bacterial microbiota composition was significantly closer to that of their mother, implying an intrauterine, placental or amniotic fluid origin of such bacteria. Moreover, bacterial communities differed according to the delivery mode. The high colonization by *R. ornithinolytica* in this study may be related to maternal colonization and inoculation of the neonates. However, Kigbu *et al* [21] did not evaluate maternal samples, therefore maternal source of neonates' microbiota could not be ascertained. The diversity found by Brazier *et al* [22], which differed from Kigbu *et al* [21], may not be unrelated to the fact that Brazier *et al* utilised metagenomic sequencing compared with API 20NE® used in the study by Kigbu *et al* which is less sensitive [21, 22, 25].

Recent reports suggest increasing relevance of *R. ornithinolytica* in human infections [10-12, 23]. The significance of colonization by *R. ornithinolytica* observed in this study therefore warrants a closer interrogation to ascertain if it plays any role in subsequent neonatal infections, antibiotic susceptibility or resistance, financial implication of cost of care, duration of hospitalisation and mortality. In most newborn units, aminopenicillins and aminoglycosides, are among the first line antibiotics recommended. The importance of *Roultella spp*, is that it can express virulence factors and could be 100% resistant to commonly used antibiotics [26]. The possession of chromosomal beta lactamases in *R. ornithinolytica* makes the organism resistant to aminopenicillins [27]. In addition, extended-spectrum beta lactam and carbapenem resistance have been documented, including in neonates [12]. Being an enterobacterale, *R. ornithinolytica* can potentially acquire resistant conferring genes from plasmids transferred from other enterobacterales. The role of *R. ornithinolytica* in new born infection remains unclear, however the organism have been reported as cause of both early and late onset neonatal infection, including carbapenem resistant strains [12]. The high proportion of neonatal GIT colonization in the present study is a cause of concern about the possiblity of its translocation into the blood stream and resultant blood stream infection. If this occurs, managing such neonates might be quite challenging.

As far as accessible literature have revealed, this study appears to be the first time that *R. ornithinolytica* is reported as a GIT colonizer in Nigeria. The *Roultella spp*, were initially classified as *Klebsiella spp*, but recognition of the different biochemical characteristcs led to a reclassification in 2001 [28]. However, laboratory diagnosis remains a challenege and different microbiological identification modalities have performed differently [25]. The challenge and difficulty to properly identify *R. ornithinolytica* with conventional biochemical and phenotypic tests may explain the low prevalence of *R. ornithinolytica* infections and colonization in the literature.

Recent introduction of matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) led to an increased identification of *R. ornithinolytica* in clinical specimens [23], further elucidating their increased role in clinical diseases. In another study Park *et al* [25], compared to the sequence-specific primer PCR, VITEK 2 GN ID card (bioMérieux) performed better identifying all *R. ornithinolytica* isolates correctly, while MicroScan and API 20E misidentified 92.6 % and 88.9 % of *R. ornithinolytica* isolates as *Klebsiella oxytoca* respectively [25]. However, going by the potential of API 20E to misclassify *R. ornithinolytica* as *Klebsiella spp*, it may suggest an even higher proportion than is reported in this study.

Few studies have evaluated the risk factors associated with *R. ornithinolytica* infection or colonization. The new born microbiome is known to be affected by gestational age at delivery, the mode of delivery, breastfeeding practice and antibiotic exposure amongst others [29]. Infants delivered via the vagina have gut colonization reflective of maternal vaginal flora such as *Lactobacillus* and *Prevotella* species while infants delivered by Caesarean section are colonized by epidermal rather than vaginal species, such as Clostridium, Staphylococcus, *Propionobacterium* and *Corynebacterium* [30]. Aagaard *et al* [20] observed that in the first week of life the full-term neonatal gut microbiome is largely colonized by members of the *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, and, much less, *Firmicutes* phyla. Colonization with *R. ornithinolytica* and various risk factors were evaluated in this study, gender, mode of delivery, gestational age at delivery asphyxia and duration of

labour. None was found to be associated with *R. ornithinolytica* colonization on bivariate analysis. The colonization rate over time also appeared to reduce over time to a low level of 5.7% at 14th day of life

Conclusion

R. ornithinolytica is an under-reported, human pathogen bacterium in neonates which may be a saprophytic colonizer, contaminant or may be a cause of unreported neonatal infections which are not identifiable because of current laboratory methods in developing countries. If the results of the *Raoultella* colonization is confirmed by further next generation PCR, the implications on probiotics, and synbiotics could only be imagined. Since gut microbiota plays important roles in digestive, metabolic and neurological functions as well as probiotics preparations, further evaluation of *R. ornithinolytica* in dysbiosis is necessary.

Paediatricians should be aware of the possibility of neonatal infections caused by this bacterium and associated high rates of antimicrobial resistance of *R. ornithinolytica* isolates, this may impact on antibiotic choices. Gut colonization in the neonatal period warrants further interrogation of its role in nosocomial infections and community acquired infections. Studies examining the outcome of colonized patients will provide a better understanding of the role of colonization by *R. ornithinolytica* in the newborn period.

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