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Evaluation of the association of pneumococcal conjugate vaccine immunization and density of nasopharyngeal bacterial colonization using a multiplex quantitative polymerase chain reaction assay

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ABSTRACT

Background: Nasopharyngeal bacterial colonization is a pre-requisite for developing bacterial mucosal and invasive disease. Pneumococcal conjugate vaccine (PCV) immunization of children reduces their risk of colonization by vaccine-serotypes, which could affect the biome of the nasopharynx in relation to colonization by other bacteria. This study evaluated the association of PCV immunization on the prevalence density of nasopharyngeal colonization by common, potentially pathogenic bacteria.

Methods: A multiplex qPCR assay was used to evaluate bacterial nasopharyngeal colonization by 7-valent PCV (PCV7) serotypes, non-vaccine serotypes (NVT), *Haemophilus influenzae*, *Staphylococcus aureus*, *Moraxella catarrhalis*, and *Neisseria meningitidis* in PCV7-vaccinated and PCV-unvaccinated African children at two time points.

Results: PCV7 vaccination was associated with a higher prevalence of NVT and *H. influenzae* at 9 and 16 months, respectively. While the prevalence of *S. aureus* was higher in PCV7-vaccinated children at 9 months, no difference was found at 16 months. The density of PCV7 serotypes (3.8 vs. 3.4 $\log_{10}$; $p = 0.048$), NVT (3.6 vs. 3.1 $\log_{10}$; $p = 0.018$), *H. influenzae* (4.34 vs. 3.86 $\log_{10}$; $p = 0.008$), *M. catarrhalis* (3.52 vs. 2.98 $\log_{10}$; $p < 0.001$) and *S. aureus* (4.02 vs. 3.06 $\log_{10}$; $p = 0.02$) was higher among PCV-vaccinated compared to PCV-unvaccinated children at 9 months, although, this difference diminished at 16 months of age.

Conclusion: The reduction in PCV7-serotype colonization impacted on colonization prevalence and density of other bacterial species of the nasopharynx. The clinical relevance of this needs further exploration in relation to mucosal and invasive disease outcomes, as well as for higher valency PCV vaccines.

1. Background

Invasive and mucosal disease due to bacterial pathogens such as *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus* follow on colonization of the pharynx, with bacterial and host factors contributing to the nasopharyngeal colonization patterns of these bacteria [1,2]. Vaccination of children with pneumococcal conjugate vaccine (PCV) reduces the risk of *S. pneumoniae* vaccine-serotypes (VT) nasopharyngeal colonization, but is associated with an increased detection of non-vaccine serotypes (NVT) [3]. The effect on pneumococcal colonization induced by PCV could also affect colonization patterns of other bacteria, including *S. aureus* which has an inverse association with *S. pneumoniae* colonization, particularly with the seven-valent PCV (PCV7) serotypes and *H. influenzae* which is positively associated with pneumococcal colonization [4–6].

Since bacterial nasopharyngeal colonization is the precursor to disease of the upper and lower respiratory tract, carriage analysis in PCV-vaccinated and PCV-unvaccinated children could provide a measure for predicting whether there might be changes in susceptibility to mucosal and systemic bacterial infections in these children as a result of PCV immunization [1]. While a limited number of studies have used quantitative PCR (qPCR) methods to

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investigate bacterial nasopharyngeal colonization [4,7], the majority of studies have focused on non-quantitative culture-based methods as recommended by WHO [8], which may underestimate the prevalence of pneumococcal colonization and do not allow for quantitative evaluation of bacterial colonization or the effect of PCV thereon.

In this study we expand on our previous findings were nasopharyngeal samples from PCV-vaccinated and PCV-unvaccinated children were cultured for *S. pneumoniae*, *H. influenzae* and *S. aureus* using standard culture methods and the dynamics of colonization over the first two years of life were described [9,10]. Here we explore the association between PCV7 immunization and the density of colonization by these bacteria, as well as colonization by *M. catarrhalis, S. pyogenes* and *N. meningitidis* as measured by multiplex qPCR.

2. Results

Quantitative PCR analysis involved 713(83%) of the initial 857 nasopharyngeal swabs collected from children, at two time points [11]. Demographic characteristics of the study cohorts have been reported previously [11].

2.1. Detection of bacterial carriage by culture and qPCR

There was modest concordance between qPCR and culture for the detection of pneumococcus at 9 (*kappa* = 0.59) and 16 months (*kappa* = 0.59) of age. *LytA* qPCR assay was more sensitive than culture in detecting pneumococcus in both PCV7-vaccinated (71% vs. 57%; $p < 0.001$; and 77% vs. 61%; $p < 0.001$) and PCV-unvaccinated (83% vs. 74%; $p < 0.001$; and 81% vs. 68%; $p < 0.001$) children at 9 and 16 months of age, respectively; Table1.

There was modest concordance between qPCR and culture for the detection of *H. influenzae* at 9 (*kappa* = 0.50) and 16 months (*kappa* = 0.61) of age. The qPCR assay was more sensitive than culture in detecting *H. influenzae* in both PCV7-vaccinated (66% vs. 48%; $p < 0.001$; and 72% vs. 54%; $p < 0.001$) and PCV-unvaccinated children (56% vs. 31%; $p = <0.001$; and 62% vs. 58%; $p = 0.04$) at 9 and 16 months of age, respectively; Table1.

There was also moderate concordance between qPCR and culture for the detection of *S. aureus* at 9 (*kappa* = 0.68) and 16 months (*kappa* = 0.65) of age. Amongst PCV7-vaccinated children, the qPCR method was more sensitive than culture in detecting *S. aureus* at 9 (19% vs. 13%; $p = 0.004$) and 16 months (17% vs. 11%; $p = 0.03$) of age; Table1. While there was no significant difference between the two methods in PCV-unvaccinated children at 9 months of age, the qPCR method was more sensitive than culture for the detection of *S. aureus* in PCV-unvaccinated children at 16 months of age (19% vs. 13%; $p = 0.004$).

M. catarrhalis, *S. pyogenes* and *N. meningitidis* were not tested by culture and thus could not be compared to molecular qPCR; however, their respective prevalence by qPCR were 59% and 54%; 3% and 4%; and 1% and 1% in PCV7-vaccinated; and 61% and 64%: 4% and 6%; and 3% and 2% amongst PCV-unvaccinated children at 9 and 16 months of age, respectively.

The qPCR method demonstrated high sensitivity for all bacteria for which culture was undertaken and used as a referent standard, with 96.8%, 95.6% and 82.3% of culture-positive swabs being positive by qPCR. While it was not possible to accurately calculate the specificity of qPCR due to its superior sensitivity over the gold standard culture method, 90.5%, 94.3% and 97.5% of qPCR negative swabs were also negative by culture for *S. pneumoniae, H. influenzae* and *S. aureus,* respectively. Discordant results between culture and qPCR were strongly associated with the density of carriage, with 88% of culture negative, PCR positive samples having a bacte-

Table 1
Number (%) of bacterial culture and molecular qPCR for the detection of *S. pneumoniae, H. influenzae* and *S. aureus* in nasopharyngeal swabs.

	9 month old children						16 month old children					
	PCV-unvaccinated			PCV7-vaccinated			PCV-unvaccinated			PCV7-vaccinated		
	Culture (−)	Culture (+)	Total	Culture (−)	Culture (+)	Total	Culture (−)	Culture (+)	Total	Culture (−)	Culture (+)	Total
S. pneumoniae												
PCR (−)	27 (15)	4 (2)	31 (17)	45 (26)	5 (3)	50 (29)	32 (17)	4 (2)	36 (19)	39 (24)	2 (1)	41 (25)
PCR (+)	20 (11)	130 (72)	150 (83)	31 (18)	94 (54)	125 (71)	29 (15)	128 (66)	157 (81)	25 (15)	98 (60)	123 (75)
Total	47 (26)	134 (74)	181 (100)	76 (43)	99 (57)	175 (100)	61 (32)	132 (68)	193 (100)	64 (39)	100 (61)	164 (100)
H. influenzae												
PCR (−)	78 (43.1)	2 (1)	80 (44)	59 (34)	4 (2)	63 (36)	68 (35)	5 (3)	73 (37)	42 (26)	4(2)	46 (28)
PCR (+)	47 (26)	54 (30)	101 (56)	32 (18)	80 (48)	112 (66)	14 (7)	106 (55)	120 (62)	33 (20)	85 (52)	118 (72)
Total	125 (69.1)	56 (31)	181 (100)	91 (52)	84 (48)	175 (100)	92 (48)	111 (58)	193 (100)	75 (46)	89 (54)	164 (100)
S. aureus												
PCR (−)	157 (87)	4 (2)	161 (89)	141 (81)	1 (1)	142 (81)	156 (81)	1 (1)	157 (81)	131 (80)	5 (4)	137 (84)
PCR (+)	6 (3)	15 (8)	20 (11)	11 (6)	22 (13)	33 (19)	11 (6)	25 (13)	36 (19)	15 (9)	13 (8)	28 (17)
Total	163 (90)	18 (10)	181 (100)	152 (87)	23 (13)	175 (100)	167 (87)	26 (13)	193 (100)	146 (89)	18 (11)	164 (100)