Determination of seasonal variation on prevalence of *Moraxella catarrhalis* in Irrua, Edo State

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Abstract

Due to incessant and reported cases of involvement of *Moraxella catarrhalis* in upper respiratory tract infections (URTIs) during the dry tropical season amongst individuals living in a tropical rural community, there was need to conduct a research to ascertain if seasonal variation plays a role in *M. catarrhalis* prevalence. A total of 600 samples were collected from children (0-10years) and adults (20-60years), these were examined for presence of the organism. Samples collected were sputum, nasal discharge and ear swab, 300 from children (100 of each specimen) and another 300 from adults over a 3year period (2011-2013) with actual emphasis on seasonal variation. The samples were subjected to standard microbiological procedures with further confirmation employing culture and polymerase chain reaction (PCR) tests. The PCR results yielded 22 isolates while that of culture was 19 *M.catarrhalis* isolates which was statistically not significant at P>0.05. More of the organisms were recovered from children 13(3.7%) while adults produced 9(3.3%) pure isolates. Seasonal variation was indicated when a greater number of the organisms were encountered in the dry season (18) 82% than in the wet season (4)18% which was statistically significant at P<0.05. It can be concluded that with the high prevalence of *M.catarrhalis* in both children and adults during the dry dusty harmattan months, there is need to step up public enlightenment campaign on the epidemiology of *Moraxella catarrhalis* infections.

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Introduction

Moraxella catarrhalis was regarded as one of the non gonococcal, non-meningoccal *Neisseriae* considered to be members of the normal floral □Verduin *et al.*, 2002. *M. catarrhalis* strains tolerate lower temperature (28°C) when compared to other *Neisseria* species, which grow at 35°C [2]Knapp's, 1988. Seasonal variation has also been linked to high rate of *Moraxella catarrhalis* isolation due to significant nasopharyngeal carriage rates in the dry or winter period than rainy or summer season [3]Van Hare *et al.*, 1987.

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Objective:

Determination of Relationship between seasonal variation and prevalence of *M.catarrhalis.*

Materials and Method:

The study was conducted in Edo central senatorial district, Nigeria. Collection of samples for this study was carried out from individuals attending clinic in a very busy hospital facility in Esan central LGA after due ethical approval.

A total of six hundred patients (600) patients were selected, 300 children (0-10yrs) and 300 adults (20-60yrs) employing the formula of Dean *et al* (1995). Sputum, ear and nasal swabs (100 of each from both subjects) were aseptically collected with sterile swab sticks from males and females attending out patients Department. Sample

collection was from January 2011 to December 2013, with emphasis on seasonal variation, that is dry (October March) and wet (April September) seasons. The samples were transported to the laboratory for microbiological analyses using peptone water to maintain the swabs moist and placed in geostyles (insulated cold boxes containing frozen ice packs). The middle ear, sputum and nasal swab samples were streak inoculated onto plates of MacConkey Agar and Blood Agar to which vancomycin (3µg/ml), colistin (6µg/ml) and trimethoprim (3µg/ml) had been incorporated by adding into the liquid medium after sterilization and incubated aerobically for 28°C for 24 to 48hours in a 5% CO_2 atmosphere. Colonies were sub-cultured onto Petri dishes containing chocolate agar and incubated as described above. Routine conventional laboratory techniques were all carried out using the methods described by [4]Cheesebrough, 2006. **Further identification of *M. catarrhalis* was done employing the tributyrin test.** This is a differentiation test between *Branhamella (Moraxella)* and *Neisseriae*. The test principle is an enzyme hydrolysis of tributyrin. Final evaluation was made after 20hrs with the color of the suspension turning yellow, indicating a positive reaction (tributyrin^{tve}), a negative reaction will give a red coloration. The seasonal variation pattern was divided into the dry- harmattan season (October-March) and wet-rainy season (April-September).

Statistical Method: Data was subjected to the chi square test with a probability of $P<0.05$ (level of significance).

Results: Table 1.1 shows the laboratory characteristics used in the identification of *Moraxella catarrhalis*, with the growth on blood agar made selective by addition of trimetoprim, colistin and nalidixic acid. The Nitrate reduction test and superoxide test aided differentiation of *Moraxella catarrhalis*, from *Neisseriae* species and other close family members. DNase positive test served as an indicator for *Moraxella catarrhalis* likely pathogenic status. The total number of specimens collected from the different subjects as well as number of positive isolates (n) is shown in Table 1.2. Conventional identification procedures (culture/microbiological techniques) and polymerase chain reaction (PCR) analysis were applied to the samples and the results revealed a slightly higher identification result with PCR 22(3.7%) against culture 19(3.3%) which was statistically not significant $P > 0.05$ (Table 1.3)

Table 1.4 shows a high prevalence rate of *Moraxella catarrhalis* from ear swab specimens (5%), sputum (3.5%) and nasal swab (2.5%) out of the 22 pure isolates obtained. Highest rate of *Moraxella catarrhalis* isolation was in children (4.3%) than adults (3%). The seasonal prevalence of the organism is shown in Table 1.5a &1.5b, with the dry-harmattan months (November-January) having the highest prevalence rates in children (16%, 16% and 12%) while in adults the peak period was in November and December (12% in both) when compared to the rainy season of June and July (4%).

Table 1.1: Laboratory Characteristics used in the identification of *M. catarrhalis*

Characteristics		Result
Colony Morphology	:	Non-Pigmented, Opaque, Smooth, does not adhere to agar.
Growth on Blood agar (Made Selective by adding Colistin, vancomycin & Trimethoprim	:	Positive
Growth on nutrient agar	:	Positive
Gram Stain	:	Gram negative diplococci
Oxidase	:	Positive
Deoxyribonucleic (DNAase)	:	Positive
Nitrate reduction test	:	Positive
Sugar fermentation test With glucose, maltose, Lactose and sucrose	:	Negative
Superoxide Test (30%	:	Positive
Catalase test (3%)	:	Positive

TABLE1.2: Total number of specimens collected from subjects.

S U B J E C T S

Specimens N(n)	Children (0-10yrs) N (n)	Adults (20-60yrs) N(n)	Total +ve	%
Sputum	100(4)	100(3)	200 (7)	3.5%
Ear swab	100(6)	100(4)	200(10)	5%
Nasal swab	100(3)	100(2)	200 (5)	2.5%
Total	**300(13)**	**300(9)**	**600(22)**	**3.7%**

N = Number of Samples
n = number of positive isolates.

Table 1.3: Comparism of Culture against PCR Results.

Organism	Culture (n=600)	PCR (n=600)
M. catarrhalis	19(3.2%)	22 (3.7%)
X^2 2.45	P>0.05 (not sig)	

Table 1.5a: Seasonal Prevalence of *M.catarrhalis* in Adults.

YEAR								
2011			2012		2013		TOTAL	
MONTH	Tested N	+VE N(%)	Tested N	+VE N(%)	Tested N	+VE N(%)	Tested N	+VE N(%)
JAN	10	-	10	1(10)	5	-	25	1(4)
FEB	10	1(10)	10	-	5	-	25	1(4)
MAR	10	-	10	-	5	-	25	-
APR	10	--	10	-	5	-	25	-
MAY	10	-	10	-	5	-	25	-
JUN	10	-	10	-	5	-	25	-
JUL	10	-	10	1(10)	5	-	25	1(4)
AUG	10	-	10	-	5	-	25	-
SEPT	10	-	10	-	5	-	25	-
OCT	10	-	10	-	5	-	25	-
NOV	10	1(10)	10	1(10)	5	1(20)	25	3(12)
DEC	10	1(10)	10	2(20)	5	-	25	3(12)
	120	**3(2.5)**	**120**	**5(4.2)**	**60**	**1(1.7)**	**300**	**9(3)**

Table 1.5b: Seasonal Prevalence of *M.catarrhalis* in Children.

YEAR								
2011			2012		2013		TOTAL	
MONTH	Tested N	+VE N(%)	Tested N	+VE N(%)	Tested N	+VE N(%)	Tested N	+VE N(%)
JAN	10	1(10)	10	1(10)	5	1(20)	25	3(12)
FEB	10	-	10	-	5	-	25	-
MAR	10	-	10	-	5	-	25	-
APR	10	-	10	-	5	-	25	-
MAY	10	-	10	-	5	-	25	-
JUN	10	-	10	1(10)	5	-	25	1(4)
JUL	10	-	10	1(10)	5	-	25	1(4)
AUG	10	-	10	-	5	-	25	-
SEPT	10	-	10	-	5	-	25	-
OCT	10	-	10	-	5	-	25	-
NOV	10	1(10)	10	2(20)	5	1(20)	25	4(16)
DEC	10	2(20)	10	1(10)	5	1(20)	25	4(16)
	120	**4(3.3)**	**120**	**6(5)**	**60**	**3(5)**	**300**	**13(4.3)**

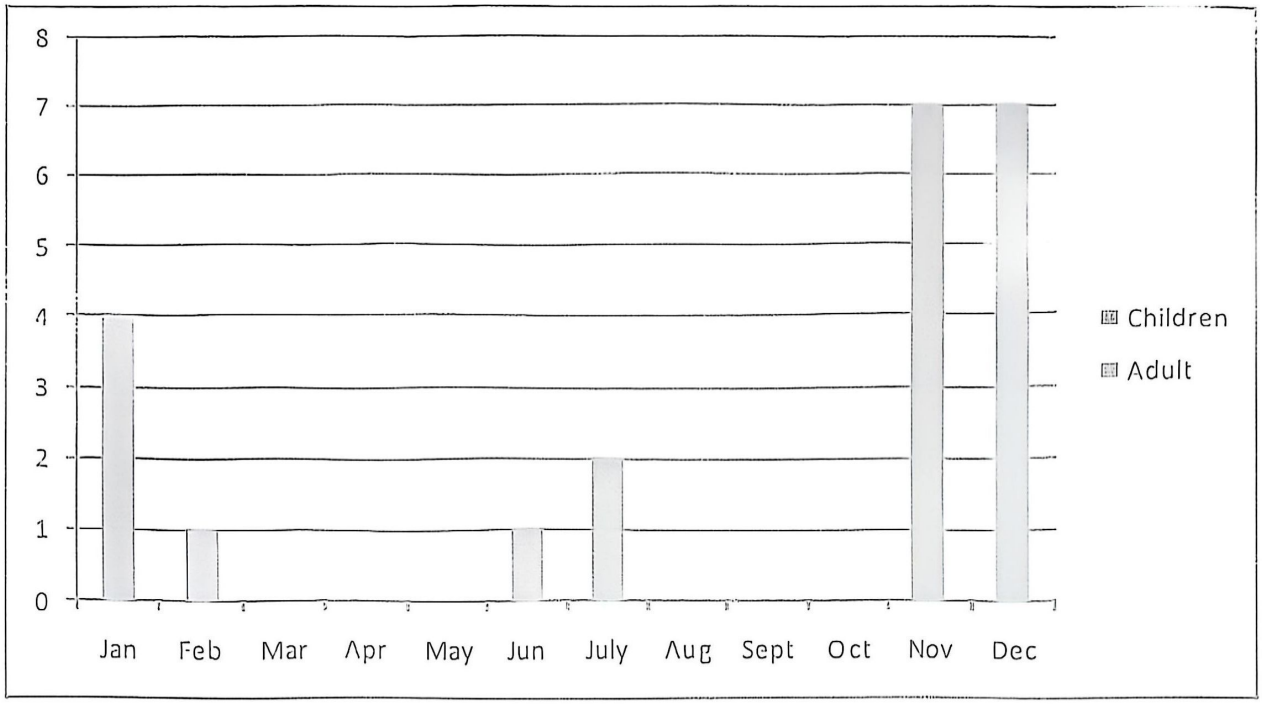

Fig 1: Cumulative seasonal occurrence of *M.catarrhalis* in adults and children.

Discussion:

Over the past decade, *Moraxella (Branhamella) catarrhalis* has emerged as an important human pathogen. The bacterium is a common cause of otitis media in children and lower respiratory tract infections in adults with chronic obstructive pulmonary disease □Murphy, 2000.

Strains were confirmed phenotypically by conventional carbohydrate assimilation profiles, tributyrin hydrolysis, Gram stain colony characteristics, and growth on selective blood agar, β-lactamase production and PCR assays [2]Bell *et al.*, 1998.

Out of the six hundred (600) specimens collected over a period of 3 years from children and adults of both sexes, 22(3.7%) positive isolates were detected from PCR analysis while culture technique detected 19(3.2%) *M.catarrhalis* isolates from the 22 isolates detected by PCR. This result is at variance with the work of [3]Guclu *et al.*, (2005) who obtained a 62.6% isolation rate of *Moraxella catarrhalis* using PCR against 10.3% using conventional culture techniques.

The seasonal prevalence of the organism was observed to be statistically significant ($P < 0.05$) during the dry harmattan (winter) months of November, December and January (16%, 16% and 12%) for children and (12%) for adults as compared to the rainy seasons (summer) of June/July with occasional isolation of 4%. This is in agreement with the work of □Wood *et al.*, (1996) and □Murphy (2000) who both reported that *Moraxella catarrhalis* carriage rates were significantly higher in winter than in summer (rainy/sunshine) period. This could be attributed to presence of infectious nuclei that are easily carried in the air during the dry harmattan periods due to heavy winds and light dust which is a common occurrence in the sample geographical sites. While the higher prevalence rate during the November/December period could be attributed to the heavy client flow at the health facility (as documented in the patient register) during the dry season, a lot of agricultural harvest and bush burning also occurs during the dry season in parts of Edo State.

This data is in line with the work of [5]Wirth *et al.*, (2007) that assembled a collection of 268 *M. catarrhalis* of diverse geographical origins and did not detect any

obvious geographical clustering or evidence for bio-geographic specificity.

Conclusion

The study has shown that, dry dusty wind which is a common occurrence in November-February and cold air enhances spread of *M. catarrhalis* amongst individuals in the tropics and not only in cold climatic regions of the world. Therefore extra precautionary measures should be adopted during the dry tropical season.

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