ANTIBIOTIC SENSITIVITY PATTERN OF MORAXELLA CATARRAHALIS ISOLATED FROM PREGNANT WOMEN ATTENDING ANTE NATAL CLINIC AT IRRUA SPECIALIST HOSPITAL, IRRUA, EDO STATE.

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ABSTRACT

Due to reported incidence of antibiotic resistance, this study examines the prevalence and sensitivity pattern of *Moraxella catarrahalis* among pregnant women with repeated complaints of ear and sinus. A total of 300 ear swab samples were collected from pregnant women at different trimesters (first trimester (18), second trimester (102) and third trimester (180)). The samples were cultured, and the isolates identified and characterized using their cultural, biochemical, and microscopic characteristics. Out of the 300 samples, 36 isolates of *Moraxella catarrhalis* were obtained. Antimicrobial susceptibility test on the isolates showed a high sensitivity pattern to Amoxicillin-clavulanate (100%) followed by Sparfloxacin and Ciprofloxacin (91.7%) each, Augmentin and Ofloxacin (83.3%) each, while Chloramphenicol was (75%). The isolates exhibited a very high resistance (100%) to Penicillin while β-lactamase test revealed that all isolates of *M. catarrhalis* were β-lactamase producers. The highest percentage of isolates was found in women within the third trimester (13.3%) and lowest in the second trimester (9.8%). Other organisms isolated were *diptheriods* (39.3%), *Streptococcus pneumoniae* (35.3%) and *Staphylococcus spp* (13.3%). This study reveals that these organisms are prevalent during pregnancy and showed varying susceptibility to antibiotics. It is therefore necessary to further evaluate the consequence of these organisms on pregnancy.

Keywords: Irrua, Antibiotic, Sensitivity, *Moraxella catarrhalis*, pregnant women.

Received: 10th June, 2012 **Accepted: 20th July, 2012** **Published: 31st July, 2012**

INTRODUCTION

Moraxella (Branhamella) catarrhalis formerly called *Neisseria catarrhalis* or *Micrococcus catarrhalis* is a gram-negative, aerobic diplococcus usually found as a normal commensal of the upper respiratory tract (Johnson et al., 1981). However, hospital outbreaks of respiratory disease due to *Moraxella catarrhalis* have been reported, thereby establishing the bacterium as a nosocomial pathogen (Patterson *et* al., 1988; Richards *et al.*, 1993). The bacterium is now implicated as a causative agent of upper respiratory tract infections in healthy children and elderly people (Doern, 1996; Catlin, 1990). Severe infections such as pneumonia, endocarditis, septicemia and meningitis have also been associated with immune-compromised hosts (Doern, 1996; Catlin, 1990).

Transmission of this organism is said to be through direct contact with contaminated droplet secretions (Murphy *et al*., 1998). Strains of *Moraxella catarrhalis* produce beta-lactamase and are differentiated from other *Neisseria* by its lack of carbohydrate fermentation and production of deoxyribonuclease (DNase) (Brooks *et al*., 2004). The bacterium is sensitive to antibiotics such as cefixine, ciprofloxacin, ofloxacin, chloramphenicol and amoxicillin-clavulanic acid (Felmingham and Druneberg, 2000).

Although much lower in healthy adults 1.3% (Digiovanni *et al*., 1987), the carrier rate of *Moraxella catarrhalis* in children is about 75% (Varon *et al*., 2000). This inverse relationship between the age and colonization has been established since 1907 (Arkwright, 1907; Ejlertsen *et al.*, 1994). Worrisome is the fact that *Moraxella catarrhalis* was once considered a normal commensal (Johnson et al., 1981) but now implicated in several

diseases and as such, a threat (Doern, 1996; Richards *et al.*, 1993; Catlin, 1990; Patterson *et* al., 1988). Despite the relationship between age and this gram-negative bacterium, its incidence in pregnancy lacks adequate literature though pregnant women are often times classified among the immune- compromised individuals. It is therefore imperative to investigate the prevalence of this gram-negative aerobic diplococcus in pregnancy, which is an important physiological state of the life of a female.

The aim of this study therefore, is to determine the involvement of *Moraxella catarrhalis* amongst pregnant women presenting with ear discharge, persistent earache and sinus discharge. This is with the objective of determining the susceptibility pattern of isolates to classical antibiotics and possible production of beta lactamase.

MATERIALS AND METHODS

Study Area: The study was conducted at the Irrua specialist Teaching Hospital (ISTH) Irrua, the teaching Hospital of the Ambrose Alli University and located in Esan Central Local Government Area of Edo State Nigeria.

The local Government Area is bounded by Uromi in Esan North East Local Government Area (5Km) and 3 Km from Ekpoma in Esan West Local Government Area when approaching from the state capital (Benin City). The Area has a projected population of 186,313 according to NPC (2006). The indigenous language is Esan while the people are refers to as Ishan.

Study population: The study targets all pregnant women (at different trimesters of pregnancy) attending antenatal clinic at the ISTH, Irrua. According to WHO (2005), they make up 5% of the total population of the total population of the study location (Esan Central Local Government Area, Irrua).

Inclusion criteria: Women registered for antenatal clinic as at the time of the study.

Exclusion criteria: Pregnant women attending antennal clinic but were on prescribed therapeutic medication or hospitalization.

Ethical consideration: Ethical approval was sought and given by the research and Ethic Committee of ISTH, Irrua while informed consent was obtained from willing participants.

Duration of study: the study was conducted within a four month period (February to May, 2012).

Study Design: The study adducted the cohort study design.

Method of sample collection: A total of three hundred (300) ear swabs were collected from pregnant women (at different trimester of pregnancy) attending antenatal clinic at ISTH, Irrua, Edo State, Nigeria. The samples were collected by a clinician using sterile swab sticks (Evepon Industries Limited, Nigeria) and transported immediately to the laboratory in geostyles (vaccine carriers) containing frozen ice-packs. Choice of specimen was based on complaints of ear discharge and pain as well as reported incidence of otitis media in pregnant women irrespective of the age of pregnancy.

Method of sample analysis: The samples were inoculated onto MacConkey agar, thereafter discrete colonies were sub cultured onto Nutrient agar. The bacterial isolates were identified using standard bacteriological and biochemical procedures, including examination of the colony appearance like opacity, elevation, size, shape, hemolytic activity, color as well as cell micro morphological features and carbohydrate assimilation as described by Cheesbrough (2004).

Biochemical procedures included catalase test, coagulase, oxidase test, Tributyrin test, DNase test, and Optochin differentiation tests for all alpha hemolytic colonies on Tryticase Soy Agar (TSA) with 5% sheep blood. Isolates that tested positive for DNase were further sub cultured onto blood and chocolate agar made selective by adding trimethoprim (10µg), vancomycin (6µg) and colistin (5µg), which were reconstituted according to the manufacturer's specification and sterilized at 121^{o}C for 15 mins.

The plates were incubated at 37^{o}C for 24 hours, under 5% CO_2 atmosphere as described by Slack (2004). Suspected isolates were subjected to the "Hockey Puck" test which involves pushing the isolates around on the agar plate with a stick (a manual confirmatory test for *M. catarrhalis*). Smears were made from the colonies on clean microscope slides for Gram staining reaction. Antibiotic susceptibility pattern was determined using the Kirby- Bauer disc diffusion method, which makes use of thin wafers impregnated with antibiotics and transferred to Mueller Hinton agar. The method of Akinjogunla and Eghafona (2010) was adopted for interpretation of measurement of sensitivity or resistance. Beta-lactamase detection was achieved with the aid of

nitrocephin disc containing a chromogenic substance, which changes color from yellow to red for positive reaction.

Data analysis: Statistical analysis was done using the student "t" test to calculate probabilities and determine level of significance. A p-value of less than or equal to 0.05 ($p \leq 0.05$) was considered to be statistically significant.

RESULTS

Table 1 shows the distribution of *Moraxella catarrhalis* isolates with respect to the different trimester of pregnancy. Although 36 (12.0%) samples were positive to *Moraxella catarrhalis* among the 300 samples examined, however, 2 (11.10%) isolates were in the 1st trimester while 10 (9.80%) and 24 (13.30%) were in the 2nd and 3rd trimester respectively.

Table 2 shows the percentage occurrence of other organisms isolated. They include Diphtheriods 118 (39.30%), *Streptococcus pneumoniae* 106 (35.30%) and *Staphylococcus* species 40 (13.30%).

The antibiotics susceptibility testing showed the antibiogram of *M. catarrhalis* to be most sensitive to sparfloxacin 11 (91.70%), followed by ciprofloxacin 10 (83.30%), Augmentin 10 (83.30%), ofloxacin 9 (75.0%) and chloramphenicol 9 (75.0%). Penicillin was resistant 12 (100.0%) indicating that the isolate produced β-lactamase.

DISCUSSION

The 12% prevalence rate of *Moraxella catarrhalis* recorded in this study does not differ markedly from the work of Akinjogunla and Enabulele (2010) and Ekpo *et al.*, (2009) who both observed a prevalence of 18.8% and 10% in 212 samples obtained from hospitalized patients within Uyo metropolis of Nigeria. Though the number of isolates in the third trimester was highest, there was no statistical significance ($P<0.05$) in all stages of pregnancy, which is attributed to the fact that the carriage rate of *M.catarrhalis* is higher in immuno-compromised individuals (Doern, 1996; Catlin, 1990). Other established pathogens such as, *Streptococcus pneumoniae* (35.3%) and *Staphylococcus* spp (13.3%) was isolated. This result is in agreement with Wald's report, which isolated similar organisms in 30 – 40% of patients screened for *M. catarrhalis* (Wald, 1998).

Antibiotics susceptibility test showed that *M. catarrhalis* was highly sensitive to Amoxicillin-clavulanate (100%), followed by Ciprofloxacin and Sparfloxacin (91.7%), Ofloxacin and Augumentin had sensitivities ≥80%. This result shows that individuals that cannot tolerate any of the first three drugs can also use either of the last two since they showed obvious therapeutic effect against the organism.

Table 1: The distribution of *Moraxella catarrhalis* isolates with respect to the different trimester of pregnancy

Trimester	No of sample	No *of M. catarrhalis* isolated (%)
1st	18	2 (11.10%)
2nd	102	10 (9.80%)
3rd	180	24 (13.30%)
Total	300	36 (12.0%)

Table 2: Percentage representation of other organisms isolated

Micro organisms	No Isolated	% Occurrence
Diphteroids	118	39.30
S. pneumoniae	106	35.30
Staphylococcus spp	40	13.30
Total	264	88.0

Table 3: Antibiotic susceptibility pattern of *Moraxella catarrhalis*

Antibiotic (µg/ml)	Sensitive E (%)	Zone of Inhibition (mm) S	Resistance (%)	Zone of Inhibition (mm) R
Chloramphenicol (5)	27 (75.0%)	19mm	9 (25.0%)	7mm
Ciprofloxacin (30)	33 (91.70%)	21mm	3 (8.30%)	8mm
Ofloxacin (15)	30 (83.30%)	19mm	6 (16.70%)	6mm
Sparfloxacin (30)	33 (91.70%)	20mm	3 (8.30%)	8mm
Amoxicillin-Clav (10)	36 (100.0%)	20mm	0 (0.0%)	6mm
Augmentin (15)	30 (83.30%)	20mm	6 (16.70%)	8mm
Cotrimoxazole (30)	24 (66.70%)	21mm	12 (33.30%)	7mm
Gentamycin (10)	18 (50.0%)	18mm	18 (50.0%)	7mm
Pefloxacin (10)	20(55.6%)	20mm	16(44.4%)	7mm
Streptomycin (30)	18(50%)	20mm	18(50%)	7mm
Penicillin (10)	0(0%)	22mm	36(100%)	6mm

Zone diameter of Inhibition; R (resistance): ≤ 10mm; S (sensitive): ≥ 14mm

These results are in accordance with the findings of (Felmingham, and Druneberg, 2000), who reported sensitivity ≥80% for these antibiotics. *M. catarrhalis* isolated were resistant to penicillin indicating that treatment with the penicillins will not be effective. This is due to the involvement of isolates with high β-lactam activity within the sample population, this is in agreement with the findings of Bootsma *et al*., (1996) and Fung (1992), who worked on strains that produced β-lactamase.

The finding from this research shows susceptibility to infections associated with *M. catarrhalis* in all stages of pregnancy. It is therefore suggested that early ante natal care should commence as soon as pregnancy is established. Drug prescription regimen by clinicians should be based on outcome of antibiotic sensitivity tests. Public enlightenment on the biology of infections in which *M. catarrhalis* is incriminated cannot be over emphasized.

ACKNOWLEDGEMENT

We acknowledged the staff of the Irrua Specialist Teaching Hospital (ISTH) Irrua, Edo –Nigerian who provided necessary assistance toward the success of this study.

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AUTHOR'S CONTRIBUTION

Osagie R.N., is the principal investigator and was involve in the design of the study. Oseyi F.E., was involved in sample collection while Eyaufe A.A. interpreted the microbiological techniques. Eigbefoh J. screen and classified the pregnant women. Ireye F.O. interpreted the public health implication, provided the WHO data and the statistical analysis. Amechi B.O and Eidangbe, A.P was involved in the microbiological / biochemical analysis.