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ORIGINAL ARTICLE

# The Role of the Bacterial Infections of the Nose in Etiology of Primary Atrophic Rhinitis

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## ABSTRACT

***Background:***. *Atrophic rhinitis is a chronic inflammatory condition of the nasal mucosa which remains as persistent illness and of difficult management.* ***Objectives:*** *-are to evaluate the relation between microbiological flora present in the nose and pathogenesis of primary atrophic rhinitis disease and to detect the antibiotic susceptibility of these microorganisms and detection of the prevalence of Ampc beta lactamase gene among isolated strains.* ***Methodology:*** *This study was done on samples collected from 60 patients attending the Outpatient Clinic of Otorhinolaryngology at Benha University Hospital, collected during the period from February 2019 to September 2019. Bacterial cultures from nasal crust, or discharge were done for isolation of the pathogenic bacteria and detection of their antibiotic susceptibility by Vitek- 2 system,multiplex PCR was done to detect AmpC gene in isolated strains.* ***Results:*** *Klebsiella ozeana was isolated in 24 (40%) of the patients followed by Pseudomonas aeruginosa in 12 cases (20%). Klebsiella species showed 5%, 45%, and 65% susceptibility to first, second, and third generation cephalosporins, respectively. It also showed 64% susceptibility to quinolones and 42% susceptibility to amoxycillin plus clavulanic acid. The susceptibilities of the isolated Pseudomonas aeruginosa strains to antibacterial agents were 12%, 59%, and 70% to first, second, and third generation cephalosporins, respectively, and 64% susceptibility to quinolone. From the 45 enterobactericae isolates, 21 (46.7%) were AmpC β-lactamase isolates [13/24 (54.2 %) K.ozeanea, 5/12 (41.7%), 2/6(33.3%) E. coli, 1/3(33.3%) P. mirabilis* ***Conclusion****: The bacterial infection of nasal mucosa is the main trigger in patients complaining of primary atrophic rhinitis .*

## INTRODUCTION

Atrophic rhinitis is a chronic inflammatory disease of nose characterized by atrophy of nasal mucosa, including the glands, turbinate bones and the nerve endings supplying the nose and its manifestations are nasal crusting, purulent nasal discharge, nasal obstruction, and halitosis (sense of bad smell). Atrophic rhinosinusitis may be categorized into two forms: primary (or idiopathic) and secondary.[1]

Primary atrophic rhinitis prevalence varies in different regions of the world. It is a common condition in warm countries and is more prevalant in females. Although the exact cause of primary atrophic rhinitis is unknown , many patients were found to have chronic bacterial infection of the nasal mucosa and sinuses due to a large number of organisms.[2]

The most common organism is *Klebsiella* ozaenae. but other causative organisms include *Coccobacillus foetidus ozaenae, Bacillus mucosus, Diphtheroids, Bodetella pertussis, Haemophilus influenzae, Pseudomonas aeruginosa*, and *Proteus* species. However it is still not clear whether these bacteria can cause disease or just secondary invaders, the superinfection with mixed flora in nose causes destruction of the epithelial lining and ciliostasis leading to progressive mucosal changes.[3]

Iron deficiency anemia has also been suggested as a cause of primary atrophic rhinitis. The disease is more prevalent in lower socioeconomic classes. An environmental role is supported by its prevalence in rural areas. It is seen to have a polygenic inheritance pattern in 15%–30% of cases. Out of the different causes, the chronic nasal infection and autoimmunity are the major supporters.[4]

Secondary atrophic rhinitis disease is more common in the developed world. It occurs in older aged patients who undergo multiple or invasive surgeries in nose, or exposed to irradiation, nasal trauma; in the case of nasal surgery, it is identified as the 'empty nose syndrome.[5]

The prevalence of Gram-negative bacteria which are multidrug-resistant is increased in the last years, and the bacterial strains which produce AmpC β-lactamases and/or extended spectrum β-lactamases (ESBLs) are of special importance [6] .

AmpC β-lactamases are clinically important as they may cause resistance to the following antibiotics: Penicillins, Cephalosporins, Oxyimino-Cephalosporins

(e.g., Ceftriaxone and Ceftazidime), Cephamycin for example (Cefoxitin and Cefotetan), and Monobactam antibiotics. AmpC β-lactamase activity is not affected by the ESBL inhibitor as Clavulanic acid[7]

The aim of the present study is to evaluate the relation between microbiological flora present in nasal cavity and the pathogenesis of primary atrophic rhinitis , to detect the antibiotic susceptibility of these microorganisms and detection of the prevalence of Ampc beta lactamase gene among isolated strains .

## METHODOLOGY

**I. Subjects:**

This study was performed on 60 patients who were attending at Outpatient Clinic of Otorhinolaryngology Department of Benha University Hospital during the period from February 2019 to September 2019,

***Inclusion criteria*:**

Patients who were complaining of nasal crusting, purulent nasal discharge bad odour and nasal obstruction, of different ages and sex were involved in our study.

***Exclusion criteria***:

Patients of atrophic rhinitis with history of previous surgeries in the nose, exposed to nasal trauma, and Showing features of systemic diseases which affect nasal mucosa and causing secondary atrophic rhinitis disease were excluded from our study.

Approval from the Ethics and Research Committee, Faculty of Medicine, Benha University and informed consent from the patients were taken.

**Methods**

All patients under the study were subjected to complete history taking, complete examination and clinical findings were recorded. The following investigations were done: complete blood count to detect iron deficiency anemia, total protein, bacterial culture from nasal crust, or discharge for isolation of the pathogenic bacteria and detection of their antibiotic susceptibility by Vitek- 2 system, Multiplex PCR was done to detect AmpC gene in isolated strains . Plain X-ray and computerized tomography were done to show the radiological features of primary atrophic rhinitis and to determine the associated sinus infection.

***Specimen collection:***

Nasal crust, or discharge were collected in sterile containers for isolation of the pathogenic bacteria.

**Organism identification:**

***First day:***

Wet and Gram films were done to detect the presence of bacteria then loopful from the sample was taken for culture on different media ; nutrient agar and MacConkey agar .Routine bacterial cultures are incubated at aerobic atmosphere at 37°C.

***Second day:***

Culture media were examined for the presence of bacterial growth. Subcultures were done in cases of mixed growth.

***Third day:***

Direct inoculation of bacterial suspension by the VITEK 2 microbial identification system was done to identify species of bacteria and their susceptible antibiotics.

VITEK 2 system can detect about 90% or more of gram-negative and gram positive bacilli within 3 hours.[8] The AST card for VITEK- 2 Systems is an automated test methodology depending on the MIC technique reported by MacLowry and Marsh and Gerlach[9]. The instrument can detect the bacterial growth of each well present in the card in a defined time .At the completion of the incubation cycles, the values of MIC values were determined for each antimicrobial contained on the card.

**Multiplex PCR**

***Preparation of template DNA***

1. **DNA extraction**: It was done as described by the manufacture (Thermo Scientific).
2. **DNA amplification**: The primers were designed according to [10] For CMY-1 gene the forward primer was 5-GCT GCT CAA GGA GCA CAG GAT-3 and the revers primer was CAC ATT GAC ATA G G T GTG G TG C-3, expected amplicon size 520 bp. For CMY-2 gene, the forward primer was 5-TGG CCA GAA CT G ACA GGC AAA-3 and the reverse primer was 5-TTT TC CTG AAC GTG GCT GGC-3, amplicon size was 462 bp. For ACC-1 gene, the forward primer was 5-AAC AGC CTC AGC AGC CGG TTA-3 and reverse primer was 5-TTC GCC GCA ATC ATC CCT AGC-3, the amplicon size was 346 bp. For FOX-1 gene, the forward primer was 5-AAC ATG GGG TAT CAG GGA GAT G-3 and the reverse primer was 5-CAA AGC GCG TAA CCG GAT TGG-3, the amplicon size was 190 bp .

PCR was carried out in a thermal cycler (Biometra, Germany) 50 µl volume reaction mixtures containing 1µl of each primer, 5 µl of crude template DNA, Water, nudease-free16 µl and 25 µl DreamTAq Green PCR Master Mix. (Life Technologies, Rockville, MD).

The PCR program consisted of an initial denaturation step at 94°C for 3 min, followed by 25 cycles of DNA denaturation at 94°C for 30 s, primer annealing at 64 °C for 30 s, and primer extension at 72°C for 1 min. After the last cycle, a final extension step at 72°C for 7 min was added. PCR product were analyzed by gel electrophoresis with 2% agarose .Gels were stained with ethidium bromide at 10 µg/ml and visualized by UV transillumination.

**Statistical Analysis**

The clinical data were analyzed in a report form and the data were tabulated using the computer program SPSS (Statistical Package for Social Science) version 20 .

## RESULTS

Sixty patients who were diagnosed as primary atrophic rhinitis were involved in the present study. Their ages ranged from 15 to 60 years. The male to female ratio was 1:3. Ratio of Rural to Urban population was 2.75:1. Family history was positive in 12 cases (20%). All patients having positive family history showed earlier onset of the disaese. The hemoglobin level and hematocrit was low in 33 (55%) and 28 (46.7%) patients, respectively.

The results of microbiological study are present in table 1. *Klebsiella* species were isolated from 24 (40%) of the patients then *Pseudomonas aeruginosa* was isolated from 12 cases (20%). *Klebsiella* species showed 5%, 45%, and 65% susceptibility to first, second, and third generation cephalosporins, respectively. It also showed 64% susceptibility to quinolones antibiotic and 42% susceptibility to Amoxycillin plus Clavulanic acid. The susceptibilities of the isolated *Pseudomonas aeruginosa* to antimicrobial agents were 12%, 59%, and 70% to first, second, and third generation Cephalosporins, respectively, and 64% susceptibility to Quinolones.

**Table 1: Microbiological organisms isolated from nasal swab in primary atrophic rhinitis patients (N = 60).**

| Strain | klebsiella | pseudomonas | Staph | Ecoli | Proteus | More than one organism | NO growth | Total |
|---|---|---|---|---|---|---|---|---|
| **NO** | 24 | 12 | 6 | 6 | 3 | 6 | 3 | 60 |
| **Percentage** | 40% | 20% | 10% | 10% | 5% | 10% | 5% | 100% |

Evidence of sinus infection was found in 79 (87.7%) of patients. The level system was used to evaluate the sinus infection in CT scan by Van der Veken et al.[11], staging by CT scan was classified started from Grade 0 where there is no change present to Grade IV in which there is a total opacity. The most commonly involved sinus was the maxillary sinus that was affected in 48 cases (80 %), followed by ethmoid sinuses in 41 cases (68.3%). The evaluation of the nose and the sinuses by plain X-rays and computerized tomography is shown in table 2.

**Table 2: Evaluation of paranasal sinuses from plain X-rays and computerized tomography in primary atrophic rhinitis patients (N = 60).**

| Sinus | Maxillary | | Ethmoid | | Frontal | | Sphenoid | |
|---|---|---|---|---|---|---|---|---|
| **Number of cases** | NO | % | NO | % | NO | % | NO | % |
| | 48 | 80 | 41 | 63.3 | 23 | 38.3 | 12 | 20 |

From the 45 enterobactericea isolates, 21 (46.7%) were AmpC β-lactamase isolates [13/24 (54.2 %) *K.ozeanea* , 5/12 (41.7%) , 2/6(33.3%) *E. coli*, 1/3(33.3%) *P. mirabilis*.The result is shown in table 3.

**Table 3: AmpC β-lactamase *Enterobacteriaceae* isolates.**

| Isolates | Total no. | AmpC positive isolates | % |
|---|---|---|---|
| *Klebsiella species* | 24 | 13 | 54.2 |
| *Pseudomonas aeruginosa* | 12 | 5 | 41.7 |
| *E. coli* | 6 | 2 | 33.3 |
| *Proteus mirabilis* | 3 | 1 | 33.3 |
| Total | 45 | 21 | 46.7<br>Pvalue<.001 (highly significant) |

## DISCUSSION

Atrophic rhinitis is a chronic disease of the nose with unknown etiology. It is manifested by the presence of thick crusts in the nose which caused by progressive atrophy of the nasal mucosa. The symptoms include foetor, crusting, nasal obstruction, bleeding from the nose, cacosmia or even anosmia , secondary infection, deformity of the nose, inflammation of nose and middle ear and even, in rare cases extension into the brain and its meninges. Atrophic rhinitis can be classified into primary or secondary[12]. The aim of this study is to detect the relation between microbiological flora present in the nose and their association with cases of primary atrophic rhinitis and to detect the antibiotic susceptibility of these microorganisms and detection of the prevalence of Ampc beta lactamase gene among isolated strains.

In the present study the patients age ranged from 15 to 60 years . The ratio of male-female was 1 : 3.

This coincides with the findings of Bist et al., [5] in India ,they found that the age of onset is during child-bearing period indicating a possible hormonal role. They also found that the disease is commoner in females and the ratio of the female: male was 2.5 : 1.

Our study revealed that the ratio of rural to urban populations was 2.75:1. This coincides with the finding of a study from Poland which reported that the disease is nearly absent in the well-developed countries and common in the developing and underdeveloped countries. [13]

However a study from Norway found a high incidence of iron deficiency anemia without an increase in incidence of atrophic rhinitis [14]. Our study also showed low hemoglobin in 33 cases (55%) which confirms the importance of a nutrition as a cause for primary atrophic rhinitis.

The family history was positive in 12 patients (20%) in present study. This finding supports that the hereditary factor was important in the etiology of this disease.This coincides with the findings of Bist et al.[5] They found that a family history was positive in 12 patients (13%)

Our study detected that *Klebsiella* species were present in cultures in 24 (40%) of the patients followed by the *Pseudomonas aeruginosa* species in 12 patients (20%).

In one study done on 61 Indonesian subjects complaining of atrophic rhinitis. They found 71.6% were *Klebsiella* species, 32.8% were *Pseudomonas aeruginosa* and 22.9% were *Staph aureus* **.** [14]

Another study in Thailand reported that *Klebsiella ozaena* is present in (67.4%), followed by *Pseudomonas aeruginosa* in 34.8%, *Pr. mirabilis* was found in 10.9%, and *Staph aureus* was found in 6.5%[15].

This did not coincide with results of Parameshwar Keshanagari and Rhesa Noel [3] who found that the most common bacteria isolated was *Pseudomonas aeruginosa* (72%), followed by *S. aureus* (12%) and other bacteria were *E. coli* (8%) and *Proteus mirabilus* (6%). In one case (2%) the nasal swab was sterile.

Studies by Artiles et al.[16] and Zohar et al.[17] suggest that chronic bacterial infections of nasal region may be leading to primary atrophic rhinitis. Few organisms have been cited as a causative agents such as *Pseudomonas, Proteus species, diphtheroids, Bordetella pertussis* and *Haemophilus influenzae*.

The presence of infection of the nose and the sinuses confirm the importance of chronic bacterial infection in the pathogenesis of primary atrophic rhinitis. Their role in the disease is controversial, they may present as secondary invaders. This can be confirmed only by studies on the experimental animals..[5]

Klebsiella species and other bacteria that cause sinusitis can slow ciliary movement and disrupt normal ciliary activity, this means impairing the mucociliary clearance which leads to chronic infection and progressive changes in mucosa of the nose . [18]

The antimicrobial susceptibility of these bacteria is dynamic and should be studied individually because the antibiotic use is still the basic of medical therapy in these cases [19].

In our study from the 45 enterobactericea isolates, 21 (46.7%) were AmpC β-lactamase isolates [13/24 (54.2 %) *K.ozeanea*, 5/12 (41.7%) , 2/6(33.3%) *E. coli*, 1/3(33.3%) *P.mirabilis*. This coincides with the findings of El hady et al.[20] in Egypt .they found that from a total of 148 Enterobacteriaceae isolates 85 (57.4%) were *Klebsiella* , 42 (28.4%) were *E. coli*, and 21 of them (14.2%) were *P. mirabilis* , that were collected from 148 different samples from ICU admitted patients

The common features found in the patients declare that the disease is multifactorial , bacterial infection of nasal mucosa, anemia, nutritional deficiency , and the hereditary element .

## CONCLUSION

From this study we can conclude that the initial cause of primary atrophic rhinitis is an infection of the nasal mucosa, which results in damaged ciliated epithelium and can be considered as one of the multifactors in these cases Vitek-2 system is a potentially reliable method for identification of bacteria and detection of antimicrobial susceptibility increasing of Amp C genes in the Enterobactericea emphasizes on the accurate use of these antibiotics to prevent any treatment failure.

**Conflict of interest:** None declared

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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