JBAAR
Journal of Basic and Applied Research in Biomedicine
ISSN 2413-7014

*Original article*

# Comparable Efficacy of Azithromycin versus Ceftriaxone in Eradicating H. influenzae of Pediatric Nasal Carriers in Iraq

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Received: 17-6-2019
Revised: 1-7-2019
Published: 24-8-2019

**Keywords:**
*Azithromycin,*
*Ceftriaxone,*
*H. influenzae,*
*Nasal carriers.*

**Abstract: Background**: *Haemophilus influenzae* is a major cause of many serious infections. Nasal carriage contributes to upper respiratory tract infections. Its incidence in pediatric people is well detected worldwide. The guidelines for treatment include many classes of antimicrobial agents with graded response according to many factors. **Patients and Methods:** This study was carried out on 200 pediatric patients with an age range of 1-5 years, divided equally into 2 groups. Nasal swab was taken from both groups prior to medication, looking for *H. influenzae*. Nasal carriers in each group were defined. Then, the 1st group carriers were then subjected to treatment with ceftriaxone parenterally for 5 days in a dose of 50 mg/kg/d, while the carriers in the 2nd one were treated with azithromycin 200 mg/d orally for 5 days. After the treatment course, 2 days were left, and then another nasal swab was taken from each carrier child. **Results:** The swabs taken from both groups reveled the ability of both treatment regimens to eradicate H. influenza, with slightly better effect for azithromycin. **Conclusion:** The results obtained in this study clearly demonstrated the beneficial effect of using a 3rd generation cephalosporin or azithromycin in the eradication of H. influenzae and confirmed the role of antimicrobial agents in targeting the expected upper respiratory tract infections accompanying H. influenzae nasal carriage.

Cite this article as: Al-Anbari, H., Al-Hilali, K., Sughair, MH. (2019). Comparable Efficacy of Azithromycin versus Ceftriaxone in Eradicating H. influenzae of Pediatric Nasal Carriers in Iraq. Journal of basic and applied Research in Biomedicine, 5(1): 49-54



## INTRODUCTION:

*Haemophilus influenzae* could cause many events, varying from an asymptomatic infection passing through severe invasive disease with elevated mortality rates (Barbour,1996). The colonization of the upper respiratory tract by non-specified strains and encapsulated strains of *H. influenzae* (serotypes a through f) might be affected by personal and environmental factors (Khleifat et al., 2006a; Michaels et al,1976). Most *Haemophilus* species are typical inhabitants of upper respiratory tract of humans and many animals (Granoff and Daum ,1980; Murphy et al, 1985). The species of *Haemophilus* that nearly frequently affect human being are *H. influenzae*, *H. aegyptius* (conjunctivitis) and *H. parainfluenzae*, *H. haemolyticus, H. parahaemolyticus*, *H. aprophilus*, *H. paraphrophilus* and *H. segnis* (abscesses and infective endocarditis) (Li et al ,1986; Bakir et al 2002).

During the last century, antibiotic- resistant *H. influenzae* strains have emerged, and the major resistance mechanism was the production of beta-lactamase (Lerman et al ,1979; Howard etal, 1988; Thornsberry etal,1999). The patterns of antibiotic resistance diverge in different areas in the world and have been growing, combating the therapy for diseases initiated by *H. influenza* (Zanella et al, 2002; Blosser-Middleton et al.2003; Gehanno et al,1996).

Respiratory tract infections (RTIs) have been a major cause of death in children globally. Although viruses are considered main RTI pathogens, bacteria share the responsibility for some localized RTIs, like sinusitis or pneumonia. Worldwide, Streptococcus pneumoniae and Haemophilus influenzae are the most important pathogens of RTIs. Streptococcus pneumoniae caused 800,000 deaths in children younger than 5 years 18 years ago (O'Brien et al., 2009), and H. influenzae accounted for at least 400,000 deaths 12 years ago (WHO,2006). Moraxella catarrhalis, S. pneumoniae and H. influenzae, represent top 3 bacterial pathogens, being the main cause of community-acquired RTIs (Biedenbach et al, 2001; Johnson etal,2003). Staphylococcus aureus is considered a primary cause of bacterial infections in hospital, including pneumonia and sepsis (Waters et al ,2011; Wertheim et al, 2005). Nasopharyngeal colonization with respiratory pathogens is the

ancestor to the onset of RTI (Garcia-Rodriguez etal, 2002), and can develop to primary infection or secondary superinfection by viruses inside its host (Diavatopoulos et al., 2010;). In addition, asymptomatic carriers are a predictable source of community-acquired RTIs (Wertheim et al, 2005; Garcia-Rodriguez et al., 2002; Adegbola et al, 2014). The infections by bacteria reflect physiological and histopathological alterations probably such as in the case of infection by Haemophilus influenza which is a major cause of many serious infections (Khleifat et al., 2002; Homady et al., 2002a; Homady et al., 2002b; Khleifat, 2006; Khleifat et al., 2006; Qaralleh et al., 2009). About 1 % of infants aged 1 year were shown to carry one respiratory pathogens (Garcia-Rodriguez et al, 2002). Several studies have revealed nasal carriage of bacterial pathogens in children, focusing on more than one pathogen (Adegbola et al, 2014).

**Antibiotics**

Azithromycin

This antimicrobial agent has a mechanism of action of arresting RNA-dependent protein synthesis, through its binding to the 50-S ribosomal subunit of the microorganisms, blocking peptidyl t-RNA dissociation from the ribosomes. In vitro incubation techniques showed high concentrations of azithromycin in fibroblasts and phagocytes, while in vivo trials showed that its concentration in phagocytes may be the reason that the drug distributes to the inflamed tissues. It can treat mild-to-moderate bacterial infections. Plasma levels are very low, while tissue concentrations are so higher, explaining its value in targeting intracellular organisms. The drug exhibits a long intracellular half-life (Tarawneh et al., 2009; Katzung et al, 2012; Khleifat et al., 2014; Althunibat et al., 2016).

Ceftriaxone

This antimicrobial agent is a third-generation cephalosporin. It has a broad-spectrum action, gram-negative activity; with less efficacy against gram-positive microorganisms, and greater efficacy against resistant organisms. Its mechanism of action is by binding to several penicillin-binding proteins, interfering with synthesis of peptidoglycan and consequently bacterial cell wall, arresting bacterial growth. The activity of cell wall autolytic enzymes lyses bacteria, while cell wall synthesis is stopped. It is stable against beta-lactamases (penicillinase and cephalosporinase). Around 1/3 to 2/3 of the dose is excreted unchanged in urine, and the rest is secreted through bile and, finally, in feces as inactive compounds. It binds reversibly to plasma proteins, and this binding may decrease from 95% to 85% as plasma concentrations increases (Abboud et al., 2010; Khleifat et al., 2006c; Katzung et al, 2012).

## PATIENTS AND METHODS

**Patients:**

This study was carried out on 200 pediatric patients, chosen randomly from Al-Hussainiya discrit which belongs to Karbalaa province/ Iraq, with an age range of 1-5 years, divided equally into 2 groups. A total of (200) nasal swabs were taken from children under five years old during a period extending from February to June 2017, with the aid of the working staff at the outpatient clinic of Karbala pediatric teaching hospital in Karbala, chosen from children in country side of Karbala city.

**Sample collection**

Nasal swabs were taken from both groups prior to medication, looking for H. influenzae. Nasal carriers in each group were defined. Then, the 1st group nasal carriers were then subjected to treatment with ceftriaxone parenterally for 5 days in a dose of 50 mg/kg/d, while the nasal carriers in the 2nd one were treated with azithromycin 200 mg/d orally for 5 days. After the treatment course, 2 days were left, and then another nasal swab was taken from each carrier child.

**Laboratory diagnosis of Haemophilus influenzae**

Haemophilus influenzae are pleomorphic Gram negative coccobacilli. Their laboratory diagnosis is based on growth and colony morphology in Chocolate Agar, and cell morphology on Gram staining. These are confirmed by the haemophilic character of the genus that reflects a requirement for either or both of the two factors called X and V. The X factor comprises haemin or other iron containing porphyrins and V factor comprises nicotinamide adenine dinucleotide (NAD) (Kilian, 1976).

**Culture**

Inoculate samples onto chocolate agar media, and then incubate at 37°C in aerobic atmosphere containing 5-10% $CO_2$ for 24-48 hours (Kilian, 1976; Al-Asoufi et al., 2017).

**Colony morphology on Chocolate Agar:**

Large flat, colorless to gray or opaque colonies. Colonies are 0.5 – 1mm circular, low convex, smooth, and pale grey and transparent. With a characteristic “mouse nest” odor. No hemolysis or discoloration is seen. Encapsulated strains appear more mucoid (watery) and non-capsulated strains appear as compact grayish colonies (Khleifat et al., 2006b; Kilian, 1976).

**Biochemical reactions for differentiation.**

**Confirmatory test**

Inoculate a single suspected colony from chocolate agar onto Mueller Hinton agar plates. Place

commercially available X, V, and XV factor discs/strips on streaked plates. Incubate plates at 37°C in 5-10% CO2 atmosphere for 18-24 hours. Observe growth around the discs and H. influenzae will only grow around the combined XV (Zeidan et al., 2013; Jones, 1982).

**Serological identification (serotyping) of *Haemophilus influenzae***
(Slide agglutination test):

Agglutinating antisera for serotypes "a" to "f" are available commercially. Such sera contain antibodies directed towards somatic antigens present in patient's sera which result in agglutination. Applying one drop of normal saline on a slide and make a homogenous suspension with a single suspected colony of *H. influenzae*. Adding one drop of specific antiserum and mix thoroughly. Observe for agglutination (visible clumping) within 1 minute. A visible clumping within 1 minute is indicative of a positive reaction (Koneman et al, 1989).

**Antimicrobial susceptibility testing and sterilization methods**
Anti-microbial sensitivity testing of *H. influenzae* in Chocolate Agar, by disk-diffusion method. Moist heat sterilization was used to sterilize media and some solutions (which are not affected by heating) using autoclave under 15 bar/in² pressures at 121 °C for 15 minutes, while dry sterilization was used to sterilize glassware at 160-180 °C for 3-2 hrs. For solutions which may be denaturized by heat, filtration using 0.22µm diameter Millipore filters was used (Kilian, 1976; Qaralleh et al., 2009; Qaralleh et al., 2010; Majali, et al., 2015).

## RESULTS

Table 1 showed the demographic data of participants, there is non-significant difference between the two groups of this study concerning sampling, age and male to female distribution.

Table 1: Demographic data of participants.

| Groups | n | age | M:F | Treatment |
|---|---|---|---|---|
| Group1 | 100 | 4-54[a] months | 69:31 | ceftriaxone parenterally for 5 days in a dose of 50 mg/kg/d |
| Group2 | 100 | 8-60[a] months | 62:38 | azithromycin orally for 5 days in a dose of 200 mg/d |

Superscript (a) denotes non-significant difference.

Result obtained in this study showed that the incidence rate of H. influenzae serotype (b) were 25% and 23% in group 1 and 2 respectively, after the treatment courses were finished, 2 days were left, and then another nasal swab was taken from each carrier child. The 2nd swab results interpreted the following:

Except for 2 carriers, all the carriers from the 1st group were cured by ceftriaxone (i.e. 92% of the carriers were cured), whereas only one carrier does not respond to azithromycin (96% of the carriers were cured).
No statistically significant difference between the two groups' results (p value is less than 0.05), table 2.

Table 2: Incidence rate of H. influenzae serotype (b).

| Groups | Positive carrier | M:F | Cure rate |
|---|---|---|---|
| Group1 | 25 (25%)[a] | 12:13 | 92% [a] |
| Group2 | 23 (23%)[a] | 12:11 | 96% [a] |

Superscript (a) denotes non-significant difference.

## DISCUSSION

Most bacterial colonization remains without symptoms, but it can progress invasive in susceptible hosts. The respiratory carriage of bacterial pathogens is more in children than in adults, particularly in kindergartens, due to the easier transmission through close contact (Garcia-Rodriguez et al,2002).

Earlier studies have shown that S. pneumoniae, H. influenzae, and M. catarrhalis might colonize in children between 1 month and 3 years (Garcia-Rodriguez et al,2002), or even at 8–10 days after birth (Leach et al,1994). In Malawi, ceftriaxone I.M. was compared with I.V. ceftriaxone and was not found to increase the mortality rate, an aspect that carries an importance in developing countries, since the I.V route might not be possible (Singleton et al,2006).

Oral antibiotics with efficacy against beta-lactamase–producing *H influenzae* include: trimethoprim-sulfamethoxazole, azithromycin, cefixime, cefuroxime, clarithromycin, and fluoroquinolones (Li et al,2017).

In China, among more than 600 respiratory or vaginal isolates from pediatric patients, showed that the rates of susceptibility to ampicillin/ sulbactam, 3rd generation cephalosporin, clarithromycin, and sulfamethoxazole - trimethoprim were 95.9%, 96.4%, 72.1%, 81.8%, and 36.4%, respectively (Li et al,2017).

In Thailand, at the largest national tertiary referral center, 1126 *H influenzae* isolates from pediatric patients, in an age range from 7 days to 6 years, throught October 2007 to June 2016. All the

isolates were susceptible to amoxicillin/clavulanate, cefotaxime, ceftriaxone, cefuroxime, and ciprofloxacin, whereas the susceptibility rate to trimethoprim/ sulfamethoxazole was 50.1% (Tribuddharat and Srifuengfung, 2017).

In a Japanese study, most *H influenzae* isolates collected from patients with acute urethritis and/or epididymitis were susceptible to ceftriaxone, fluoroquinolones, macrolides, and tetracyclines. Conversely, azithromycin treatment failures were reported in acute urethritis cases despite the evidence of azithromycin susceptibility (Deguchi et al,2017).

In children aged 2-5 years with mild to moderate acute otitis media, seven days of oral antibiotic therapy is recommended. In children aged 6 years or older with mild to moderate symptoms, 7-10 days of antibacterial therapy is considered adequate (Lieberthal et al.2017).

Empirical therapy includes ceftriaxone or cefotaxime in order to provide coverage for non-penicillin-susceptible ***Streptococcus pneumonia***, and β-lactamase-positive ***H. influenzae***. Azithromycin or erythromycin is recommended to provide the coverage for atypical pathogens in older children, and vancomycin should be considered for life-threatening pulmonary infections. Directed parenteral therapy for pneumonia and bacteremia due to ***H. influenza*** includes ampicillin for β-lactamase-negative, ampicillin-susceptible strains and ceftriaxone, cefotaxime, or cefuroxime for β-lactamase-positive strains (Bradley, 2002). Studies showed that azithromycin is beneficial for treating ampicillin-resistant and ampicillin susceptible infections of ***H. influenza*** in mice (Shuichi et al,2001).

Our results were consistent with many of the above mentioned trials concerning the efficacy of ceftriaxone or azithromycin against *H influenza*; except for two point: the first was the treatment course, which was only 5 days; while the second one was the efficacy of azithromycin which seemed to be slightly superior to that of ceftriaxone, despite the fact that there is no statistically significant difference.

In conclusion, both medications showed a powerful eradication for the target bacteria, with slightly better efficacy for azithromycin in Iraqi pediatric *H influenzae* nasal carriers.

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