

IDENTIFICATION OF NEW MEDICINES: A ROADMAP TO TREAT MULTIDRUG-RESISTANT GONORRHOEAE

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Abstract– The gram negative human specific bacteria, *Neisseria gonorrhoeae* is responsible for the spread of sexually transmitted disease gonorrhoeae. It is present globally and affecting all types of societies. Over the years, gonorrhoeae have shown exceptional flexibility in acquiring resistance against most of the antibiotics used for its treatment. At present, the first-line of therapy recommended in majority of the countries is ceftriaxone. However, recent reports have suggested emergence of resistance towards this drug, especially the ceftriaxone-resistant FC428 clone. The observed resistance in *N. gonorrhoeae* towards most antibiotics is either due to plasmid induced resistance or genomic mutation induced resistance. Moreover, the presence of multidrug efflux pump enhances the resistance of the *N. gonorrhoeae* towards a variety of drugs. To slow down the emergence of resistance towards the currently used ceftriaxone for the treatment of gonorrhoeae, the dual therapy of ceftriaxone along with azithromycin is recommended. Still, the future emergence of resistance towards both the drugs cannot be ruled out. Hence, identification and clinical trials of novel compounds is urgently required. Although, a number of compounds have been identified and clinical trials have been carried out, but all of the compounds have shown some level of inferiority relative to the currently used monotherapy drug ceftriazone or dual therapy drug ceftriaxone with azithromycin, especially for the treatment of pharyngeal infection. The present review focusses on the history of the antibiotics used for the treatment of gonorrhoeae, the mechanism of resistance towards the different drugs along with the identification of novel compounds for the future treatment of gonorrhoeae.

INTRODUCTION

Gonorrhoeae is caused by the human-specific gram negative bacteria and is second only to *Chlamydia trachomatis* in terms of prevalence of sexually transmitted infection globally (Unemo *et al.*, 2019). According to the World Health Organization, approximately 78-87 million people are annually infected by gonorrhoeae cases (Unemo *et al.*, 2019). Although, the most natural site of infection of *N. gonorrhoeae* is the genital tract, but the oropharyngeal and rectal mucosa may also be colonized and infected (Unemo *et al.*, 2019). *N. gonorrhoeae* can infect the urogenital tract of both, men as well as women. In men, approximately 40% of the urogenital gonorrhoeae cases are asymptomatic (Handsfield *et al.*, 1974), the other 60% may manifest the symptoms in the form of

urethritis and epididymo-orchitis. Whereas, approximately 50% of the urogenital infection in women are asymptomatic, the rest might show cervicitis (Van Der Pol, 2014). Although, gonorrhoeae presents mostly non debilitating symptoms, but it's infection in general increases the probability of human immunodeficiency virus (HIV) spread due to the higher load of HIV virus in the gonococcal infected urogenital tract (Unemo *et al.*, 2019). In absence of effective vaccine, the only effective gonococcal infection treatment is by using antimicrobial therapy.

In general, gonorrhea infects all type of societies and infection is found in high, middle and low income countries. In recent past, an increase in the number of gonorrhoeae have been observed, especially in developed countries with better diagnostic facilities. An increase in the number of

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infections per year can be taken from the fact that in France, a doubling in the number of cases was observed in 2013 and 2015, especially among homosexual men (men who have sex with men) (Unemo *et al.*, 2019). Similarly United Kingdom also reported a significant rise in the number of gonococcal infections. Estimates suggest an 11% rise in the number of gonococcal infection in 2013 and 2015 (Public Health England, 2016), a similar increase in the number of gonococcal infections was observed in all of Australian states, with estimates suggesting an increase in between 29%-146% in the different states (Unemo *et al.*, 2019). In the US, a 13% increase in the cases of gonococcal infections was reported in 2013 relative to 2012, with approximately 395,000 cases (Maldonado and Takhar, 2013). All these reports are reflecting longer-term trends. The observed increase in the gonorrhoea cases can be attributed to the: increased urbanization and travel, inadequate or failed treatment, poor infection detection rates (Fifer *et al.*, 2016), and a decrease in the use of condom (Paz-Bailey *et al.*, 2016). When comparing the worldwide cases, higher number of gonococcal infections were observed in the African region relative to worldwide infections, with men showing approximately double the number of cases as compared to the women each year (WHO, 2018).

Gonorrhoeae mechanism of infection

The common infection and colonization site in the urogenital tract is the epithelial cells, e.g. urethra in males and urethra, fallopian tubes and the cervix in females (Edwards and Apicella, 2004). Although, the epithelial cells are the major site of colonization, a subpopulation of the gonococci inhabits the autophagosome maturation and lysosome fusion (Lu *et al.*, 2018). After the proper growth of the gonococci in the epithelial cytoplasm, the epithelial cells dies followed by exocytosis. The *N. gonorrhoeae* is well evolved and modulates the neutrophil functionality by neutralizing the neutrophil antimicrobial activities by the expression of the defensins against neutrophils (Palmer and Criss, 2018). The bacteria resides and replicates in the immature phagosomes of the polymorphonuclear neutrophils (PMNs) (Quillin and Siefert, 2018). The potential sites of the *N. gonorrhoeae* infection includes intracellular sites (PMNs and epithelial cells) and extracellular (urethral, vaginal fluids, interstitial fluids of cervical, pharyngeal and rectal tissues, saliva as well as urine).

Gonorrhoea symptoms

Apart from the discomfort related to gonococcal infection, untreated gonococcal infection in men can result in epididymitis, urethral stricture, and in extreme case can even result in reduced fertility (Unemo *et al.*, 2019). More than 50% of the infection in women are asymptomatic, when symptoms do occur, it manifest as cervicitis in women. But, in the absence of treatment in women, it can result into complications like dysuria, lower abdominal pain, and dyspareunia. In extreme cases, it can result in loss of fertility in women (Maxwell *et al.*, 1992). In general, untreated gonorrhoea significantly increases the chances of developing pelvic inflammatory disease (PID) in females by upto 10% to 20%, and thus increasing the chances of infertility (Tsevat *et al.*, 2017). A number of untreated gonococcal infections related pregnancy complications have been reported in females including ectopic pregnancies, chorioamnionitis, rupture of membranes, spontaneous abortion and preterm birth (Liu *et al.*, 2013).

In infants, gonorrhoeae can be transferred from the infected mother to the new born at the time of birth. In approximately 30%-40% of gonorrhoeae cases, perinatal transmission is observed. In general, the perinatal transmission is predominant in developing countries due to lower healthcare facilities. The infection in infants result in the neonatal conjunctivitis (*Ophthalmia neonatorum*). If left untreated, it can result in conjunctivitis which ultimately result in scarring and blindness. (Unemo *et al.*, 2019)

History of gonorrhoeae treatment

In the past few decades, most of the antibiotics used for the treatment of gonococcal infection have lost their efficacy due to the development of resistance in *N. gonorrhoeae* (Unemo and Shafer, 2014). The antibiotics used for the treatment of gonorrhoeae in the past century are no longer effective in the treatment of gonorrhoeae i.e. penicillins, early-generation cephalosporins, sulfonamides, fluoroquinolones, macrolides, and tetracyclines.

The disease is treatable with proper medications but due to the rapid development of resistance towards nearly all the drugs used for its treatment (Unemo and Shafer, 2014), it is highly recommended to prevent the infection by exercising safe sexual practices, mainly by using condom (Unemo and Shafer, 2014). The ability of *N. gonorrhoeae* to take

gene from other commensal spp. highlights the possibility of the emergence of multi-antibiotics resistant variants thus posing serious threat to public health around the globe (Unemo *et al.*, 2019). A major cause for the emergence of the drug resistance in gonorrhoeae is the unwise use of antibiotics for the treatment of other bacterial infection as well as the treatment of gonococcal infection (WHO, 2018), this scenario is especially prevalent in the developing countries (WHO, 2018). Since the use of antibiotics for the treatment of gonorrhoeae, the bacteria has rapidly incorporated resistance towards most of the antibiotics including penicillin, macrolides (like, azithromycin), sulphonamides, fluoroquinolones, tetracyclines, and early-generation cephalosporins. The first antibiotics used for the treatment of gonorrhoeae were sulfonamides introduced in the 1930s, but within one decade, *N. gonorrhoeae* developed widespread resistance towards sulphonilamides (Unemo *et al.*, 2019). In 1943, penicillin was recommended for the treatment of gonorrhoeae. In the next decade, continuous upward dose adjustment was recommended and by the end of the decade, increasing cases of treatment failure were reported (Faruki *et al.*, 1985). In the late 1970s and 1980s, widespread resistance towards penicillin was observed due to the spread of the plasmids encoding TEM-type β -lactamases, which provide highlevel penicillin resistance (Ashford *et al.*, 1976). In the late 1960s, tetracycline was recommended as an alternative antimicrobial for gonococcal therapy, but by 1980s, the use of tetracycline had to be discontinued due to the increasing drug resistance and treatment failure due to the plasmid borne gene TetM that imparted high level of resistance towards tetracycline (Morse *et al.*, 1986). In the early 1960s, the aminocyclitol spectinomycin became an alternative recommended gonococcal treatment option, but like with other drugs, within the next two decades, widespread spectinomycin resistance was observed (Stolz *et al.*, 1975). By the 1980s, the use of spectinomycin for the treatment of gonorrhoeae was discontinued (Boslego *et al.*, 1987). Although due to its rare use after 1980s, the gonorrhoeae bacteria again become sensitive to spectinomycin. Due to the same reason, spectinomycin is still recommended as alternative therapy in a number of countries (Lee *et al.*, 2011). By mid 2000s, due to the emergence of resistant strains, the use of ciprofloxacin was removed from treatment guidelines in most countries. In the 1990s,

azithromycin was widely recommended for the treatment of gonorrhoeae, however, by late-1990s, its use was discontinued as a monotherapy, although it is still recommended in dual therapy in combination with ceftriaxone (Bignell and Unemo, 2013).

Present gonococcal infection treatment

At present, due to the development of drug resistance towards most of the antibiotics, the only successful treatment recommended in most countries is the broad spectrum cephalosporin (ESC) cefixime based therapy in injectable form. In recent years, resistance has been reported against ceftriaxone and ceftriaxone plus azithromycin dual therapy (Unemo *et al.*, 2011). The resistance is very commonly found in the international transmission of FC428 clone, which shows resistance towards ceftriaxone (Chen *et al.*, 2019). The emergence of the resistance towards ceftriaxone highlights the vulnerability of the last available first-line treatments, which further highlights the emergence of multi-drug resistant gonorrhoeae in the near future. Henceforth, dual antimicrobial therapy, mainly comprising of ceftriaxone plus azithromycin is the usual recommended treatment in most countries (Unemo *et al.*, 2019). The WHO Gonococcal Antimicrobial Surveillance Programme (GASP) have reported the worldwide spread of resistance, although higher rates were observed in North America, Europe, Asia, Latin America, Australia, and the Caribbean, whereas due to the huge data gaps in Central Asia and Africa, identification of the spread of resistance is hampered (WHO, 2018).

Multi-drug resistance in gonorrhoeae

One of the major cause of the spread of the resistance in gonorrhoeae to most of the antibiotics is because it does not affect the fitness of the organism, even in the absence of the selection pressure. This has resulted in the steady rise in the multi-drug resistance (MDR) and extensively drug-resistant (XDR). The only exception to this rule is perhaps the drug fluoroquinolone (Kunz *et al.*, 2012). In the context of gonorrhoeae, the term MDR, denotes the resistance of gonorrhoeae towards the treatment as formulated by the current guideline treatments (Tapsall *et al.*, 2009) including oral ESC, plus resistance to 2 or more of penicillins, macrolides, tetracycline, fluoroquinolones, carbapenems, and aminoglycosides. Whereas, the

term XDR is used to depict the resistance towards oral as well as the intramuscular ESCs or resistance towards 1 type of ESC and spectinomycin, with resistance to 3 or more of penicillins, macrolides, tetracycline, fluoroquinolones, carbapenems, and aminoglycosides (Tapsall *et al.*, 2009). At present, according to the WHO, the treatment guidelines should be strictly adhered to in places where the resistance towards gonorrhoeae is over 5%.

Current potential treatment compounds

In recent decades, the spread of resistance towards all major antibiotics have already raised the prospects of untreatable gonorrhoeae. To prevent such a scenario, the identification and clinical trials have not outpaced the development of the new drug (Unemo *et al.*, 2019). The slow pace for the identification and the clinical trial of novel drug will prevent the target reduction by 90% in gonorrhoeae cases by 2030 approved by the World Health Assembly in 2016. The major challenges for containing gonorrhoeae infection lies with the number of asymptomatic cases, the resistance of gonorrhoeae to nearly all major antibiotics, and the use of condoms (which alongside prevents HIV). Moreover, the development of new commercial drugs especially for infectious diseases has the added risk of market failure. Especially how the antibiotics are prescribed and sold along with the patenting of the molecule. Furthermore, there is stiff competition from the low cost generic drugs. For increasing the antimicrobial activity of the compound, there is always a need to combine two or more drug molecules, which further increases the production cost, its regulation and finally profitability. All these points towards the increased urgency for the discovery and clinical trials of the new molecules. In recent years, a number of compounds have been identified for the treatment of gonorrhoeae, although during clinical trials, only three compounds have shown promising results to varying levels i.e. solithromycin, zoliflodacin, and gepotidacin (Sun *et al.*, 2018).

Although all three compounds have shown promising result for the treatment of gonorrhoeae, but all three have shown inferiority relative to the presently used drug cefixime along with azithromycin. Hence, it still create doubt to use these novel compounds for the treatment of complicated gonorrhoeae in near future. Till then, the only successful treatment option left is the use of the present monotherapy ceftriaxone or dual

therapy ceftriaxone along with azithromycin.

CONCLUSION

In the past few decades, gonorrhoeae have become resistant to nearly all major antibiotics. Its rapid worldwide spread is raising concerns globally, with huge healthcare burden expected, especially on developing countries. Moreover, the rapid spread of the AMR among *N. gonorrhoeae* had made the generation of the drug resistant bacteria a reality. In the past century itself, *N. gonorrhoeae* had obtained resistance majorly by adaptive genomic mutations at the drug target sites, whereas for penicillin and tetracycline, the resistance is plasmid mediated. Moreover, the multidrug resistance is further enhanced by the upregulation of the multidrug efflux pump. The effectivity of the presently available first line therapy, ceftriaxone is rapidly decreasing, especially in the FC428 clone. This has infused increased urgency for the identification of novel antimicrobial compounds for the treatment of drug resistant gonorrhoeae. A number of compounds including solithromycin, zoliflodacin, gepotidacin too have inferiority during clinical trials relative to the monotherapy ceftriaxone or the dual therapy ceftriaxone along with azithromycin. Thus suggesting the best treatment for gonorrhoeae to be either the monotherapy ceftriaxone, or ceftriaxone plus azithromycin dual therapy.

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