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Review Article

Current strategies and future directions associated with to the potential of antimicrobial-resistant *Neisseria gonorrhoeae* vaccine development to rapidly reduce infection

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ABSTRACT

Gonorrhoeae, a sexually transmitted disease caused by the gram-negative diplococcus *Neisseria gonorrhoeae*, continues to be a major global health problem despite efforts to eradicate it. *Neisseria gonorrhoeae* is the second most common bacterial sexually transmitted disease in the world. This pathogen has developed resistance to all presently licensed drugs, and multidrug-resistant strains have been detected globally. The World Health Organization (WHO) currently recommends a combined therapy of ceftriaxone and azithromycin. However, when resistance to azithromycin and ceftriaxone develops, treatment failure has been documented. The only effective method to limit the spread of gonorrhoeae is protective vaccination. Previous studies investigated the immunogenicity of novel vaccine formulations based on inactivated whole-cell gonococcal microparticles loaded into soluble microneedles for transdermal delivery. Since spherical micro- and nanoparticles are biological mimics of gonorrhoeae, they are invaders of the immune system, but cannot weaken adaptive immunity. New drugs for the treatment of gonorrhoeae are important, but historical trends in resistance mean that vaccine studies must be included in all long-term plans. Proteomics and in silico methods for the detection of vaccination targets can form the basis for future success.

Keywords: Multidrug-resistance, Proteomics-driven vaccine antigen identification, Vaccine development, *N. gonorrhoeae*, Antigen variability, Immunity, Public health, Sexually transmitted

INTRODUCTION

Gonorrhoeae, a sexually transmitted disease caused by the gonococcus bacteria, remains an intractable public health problem worldwide, despite ongoing efforts to reduce health impacts [1,2]. The public health threat posed by gonorrhoeae is primarily due to the large number of asymptomatic infections, the ability of gonorrhoeae to undergo high levels of surface antigen variability, and the gradual emergence of antibiotic-resistant strains to the recommended empirical immunotherapy [3–5]. The World Health Organization (WHO) has increased the number of new therapies in the world due to the failure of treatment and the denitrification of diseases [6]. Overall, *N. gonorrhoeae* is a serious concern of public health in the world and is the most common bacterial STI in the world following *Chlamydia trachomatis*. According to the WHO, 87 million persons were diagnosed with gonorrhoeae in 2016, out of 376 million globally reported cases of sexually transmitted infections (STIs) among 15–49-year-olds [7,8], the most prevalence of which is urogenital gonorrhoeae, estimated to be higher in women (0.9%) than men (0.7%) [9]. According to a recent WHO assessment, *N. gonorrhoeae* is one of the priority areas of research for novel antibiotics [10], however, it appears that there are no new alternatives to drugs in the near future [11,12], even as new antibiotics are produced, *N. gonorrhoeae's* rapid record of resistance means it may not be effective indefinitely [13]. The emergence of antimicrobial-resistant N. gonorrhoeae, resistant to all currently available antibiotics has complicated the global increase in *N. gonorrhoeae* infections. Although WHO currently recommends dual therapy of ceftriaxone and azithromycin, many countries use a single dose of ceftriaxone or cefixime [14], nevertheless, of strains of *N.*

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gonorrhoeae to both ceftriaxone and azithromycin has been previously reported [15]. Adapting currently available antibiotics, finding innovative alternative therapies, and developing vaccines are important to prevent both the high incidence of *N. gonorrhoeae* and new resistances [16,17]. The Technical Conference on Vaccination against Sexually Transmitted Diseases convened by the World Health Organization (WHO) in April 2013 called for continued efforts to produce a gonorrhoeae vaccine [18, 19]. Modelling studies have shown that inappropriate immunizations can also provide an important health benefit, several earlier attempts to create gonorrhoeae vaccines have been developed, three in contemporary times, however only two have progressed to clinical trials. The initial whole-cell vaccination failed, as did a later and much bigger effort to develop a vaccine based on gonococcal pili, and a third based on porin protein was abandoned. These setbacks discouraged future research and fuelled fears that gonorrhoeae immunization would not be possible, these complicating matters are well-known, but extensive antigenic mutations affecting most of the major surface components of N. *gonorrhoeae* (including porin, lipooligosaccharide, Opa and pilus proteins) coupled with multiple mechanisms of complement resistance suggest that N. *gonorrhoeae* has the ability to evade whatever adaptive immune response the host develops against it. It has been suggested that the ratio of antibody to porin-plus-lipooligosaccharide to antibody to RMP (an immunogenic protein against which antibodies have a counter-effective blocking function) is protective, however, determinants of immunological defence in humans have not been characterized in the absence of a known and reproducible immune status. In this review, we look into the global epidemiology of *N. gonorrhoeae* resistance and the current state of new therapeutic and vaccine research.

LITERATURE REVIEW

Antimicrobial resistance *N. gonorrhoeae*: development and epidemiology

Neisseria gonorrhoeae has the potential of establishing antibiotic resistance mechanisms. Data on the development of Antimicrobial Resistance (AMR) suggest that *N. gonorrhoeae* had no AMR component prior to the use of current antibiotics, and that resistance was caused by widespread use and misuse of antibiotics. Ceftriaxone is currently the only viable empirical treatment option, emphasizing the significant need for further antibiotic research and development; several important efforts are underway in countries around the world to monitor antimicrobial susceptibility. Various gonococcal research companies around the world are participating in the joint surveillance initiative, and despite the slight differences in technology, each of these programs provides data and analysis of trends in antimicrobial susceptibility in treatment guidelines. In 1992, the World Health Organization (WHO) developed the Gonorrhoeae Antimicrobial Surveillance Program (GASP) with a designated regional focus to collect susceptibility data from participating countries, providing healthy regional and global treatment guidelines. However, only 77 countries submitted data in 2014, and the number of countries submitting AMR data for at least one antibiotic is declining year by year. It is important to implement the best monitoring programme around the world, but many countries are still developing gonococcal AMR surveillance programmes, but others have not tried it due to various limitations (*e.g.* laboratory capacity, funding, etc.). Data on *N. gonorrhoeae* is lacking in many countries, however the countries reporting resistance to ciprofloxacin in 2014-2016, show an overwhelming range from 97-100% resistance and azithromycin resistance was found to be at an average of 81-83%. A recent review of published studies found no evidence of ceftriaxone-R resistance, despite the lack of AMR data in 42.6% of African countries. Case reports from various countries show low utilization of ceftriaxone in Africa, with 0.5–1.1% utilization in Uganda, South Africa 0.1% and Ethiopia using no ceftriaxone at all. Overall, ceftriaxone appears to be used at a low rate of 5% in most countries around the world, with the exception of some Asian and European countries, the prevalence of reduced susceptibility to ceftriaxone is also minimal in these countries. As a result, WHO most often suggested a combination of ceftriaxone and azithromycin, nevertheless, it has recently been found that certain strains exhibit significant ceftriaxone resistance, however, this strain is rare and uncommon, reported only in Japan in 2009, France in 2010, Australia in 2013, and Japan in 2014. In addition, certain strains of this clone were found to have moderate azithromycin resistance and Multidrug Resistance (MDR) and another Ceftriaxone R clone called A2543, which is highly resistant to azithromycin, has been reported in both the United Kingdom and Australia. The spread of this trait can lead to a global antibiotic resistance catastrophe, although resistance to ceftriaxone and azithromycin is limited, three goals need to be pursued to limit the emergence of antibacterial resistant gonococci. First, treatments are tailored to the patient-specific gonococcal strain, followed by the development of innovative gonococcal drugs, and finally vaccines against gonococci. The countries in various WHO regions reporting gonococcal isolates with resistance to azithromycin and ciprofloxacin, as well as reduced susceptibility or resistance to ESC's are represented in Table 1 and Figure 1.

Table 1. From 2009 to 2014, the number of countries in various WHO regions reporting gonococcal isolates with resistance to azithromycin and ciprofloxacin, as well as reduced susceptibility or resistance to ESCs.

	Continents						
	Sub-Saharan Africa	**South-East Asia**	**Europe**	**North America**	**Central Asia and Oceania**	**East mediterranean**	**Total**
Azithromycin							
Surveillant Countries	3	6	26	7	15	1	58
<0.05 resistance	0	4	3	4	7	0	18
>0.05 resistance	3	1	21	2	2	0	29

Susceptible %	0	1	2	1	6	1	11
Ciprofloxacin							
Surveillant Countries	8	6	26	16	15	1	72
<0.05 resistance	0	0	0	1	3	0	4
>0.05 resistance	6	2	23	14	7	0	52
Susceptible%	2	0	0	0	0	0	2
Extended-spectrum cephalosporin (ESC)							
Surveillant Countries	9	6	27	16	16	3	77
<0.05 resistance	2	1	8	6	8	0	25
>0.05 resistance	1	4	15	0	6	0	26
Susceptible %	6	1	4	10	2	3	26

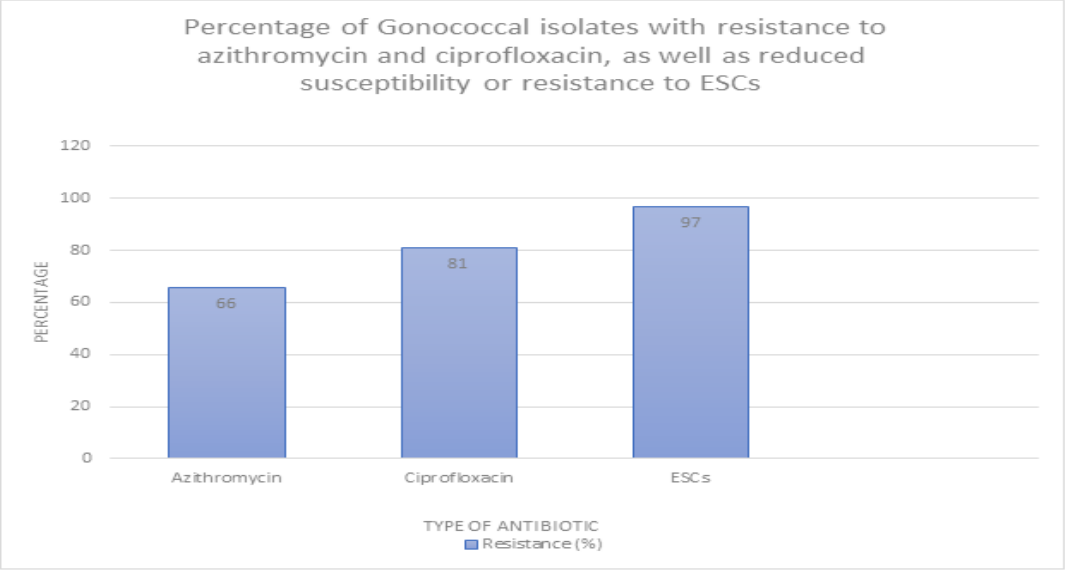

Figure 1. Percentage of Gonococcal isolates with resistance to azithromycin and ciprofloxacin, as well as reduced susceptibility or resistance to ESCs from the identified studies.

Review of current treatment recommendations for *N. Gonorrhoeae*

The introduction of each respective antibiotic, sulphonamides (1930's), penicillin (1940's), Ceftriiaxone (1960's), Azithromycin (mid 1980's), Ciprofloxacin (late 1980's), and early 2000's the introduction of combined therapies, has been due to a constant resistance development in the target organism see Figure 2. WHO currently recommends dual therapy with Extended Spectrum Cephalosporins (ESC's), primarily injectable ceftriaxone and azithromycin, for empirical treatment of gonorrhoeae however, some countries are switching to ceftriaxone immunotherapy. Due to the increasing incidence of azithromycin resistance and dual therapy failure, the Centres for Disease Control and Prevention (CDC) excluded azithromycin from recommended treatment in December 2020 and increased the recommended dose of ceftriaxone from 250 mg to 500 mg intramuscular injection. In the United Kingdom, two incidences of gonorrhoeae treatment failures related with dual therapy were recorded in 2014 and 2018. Treatment failures are most commonly associated with pharyngeal infections, which are a major source of infection despite the fact that they are mostly asymptomatic and hence mostly found by screening. Reports of gonorrhoeae treatment failures due to dual antibiotic therapy are dire warnings that the era of untreatable gonorrhoeae is approaching, and public health strategies are also vital in the treatment and prevention of antimicrobial-resistant *N. gonorrhoeae* infections, with several listed in the WHO's Global Action Plan against AMR in N. *gonorrhoeae.* A successful public health surveillance system that can quickly detect drug-resistant *N. gonorrhoeae* infections may enable targeted behaviour in response to community outbreaks. Improving the diagnosis of *N. gonorrhoeae's* AMR not only helps treat resistant infections, but also helps identify individuals who are at increased risk of treatment failure and who will benefit from follow-up treatment tests. In addition, increasing the diagnosis of resistant diseases allows for more focused contact tracing, which can lead to the identification of new diseases and reduce the spread of resistant infections. Improve understanding, awareness, and advocacy of antimicrobial resistance among physicians, public health authorities, policy makers, and the general public of *N. gonorrhoeae* can play an important role in the treatment and prevention of illness.

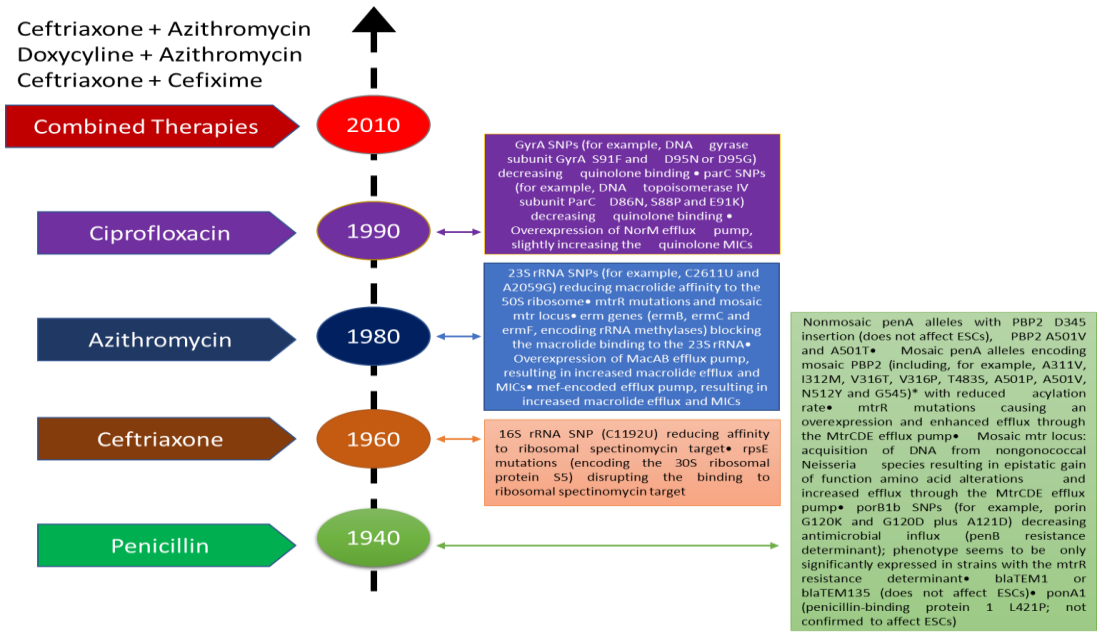

Figure 2. Empirical treatment for gonorrhoea and the establishment of AMR in *Neisseria gonorrhoeae*. The duration denotes the time period from when the therapy was first utilized until clinical and/or *in vitro* resistance threatening the efficacy of that specific antimicrobial therapy appeared, along with a description of the target protein targeted by the corresponding antibiotic.

Antibiotic repurposing

As untreatable gonorrhoeae becomes a reality, the need for new treatment options has become an urgent concern, as a result, the scientific community has turned to testing new combinations of current antibiotics as the fastest approach to tackling multidrug-resistant superbugs. In this sense, previous studies have demonstrated the feasibility of including sitafloxacin, a new-generation broad-spectrum fluoroquinolone used primarily for respiratory infections, as part of the dual anti-gonococcus regimen. Sitafloxacin was studied and evaluated using a worldwide gonococcal panel of 250 isolates and showed rapid bactericidal activity in the Minimum Inhibitory Concentration (MIC) range of 0.001 to 1 mg/L (10). In a comparable vein, another study looks at sitafloxacin and 5 different fluoroquinolones in against ciprofloxacin-resistant *N. gonorrhoeae* isolates. Sitafloxacin had more *in vitro* efficiency than the alternative 5 fluoroquinolones, with a MIC variety of 0.03–0.5 mg/L in against to ciprofloxacin-resistant bacteria. These findings guide using sitafloxacin in dual antimicrobial remedy together with delafloxacin, an antimicrobial agent presently being researched for the antimicrobial action against gonorrhoeae. Another study examined delafloxacin's activity against 117 lines of *N. gonorrhoeae*, the MIC observed was 0.001–0.25 g/mL, which is bigger than that of ciprofloxacin, penicillin, tetracycline, azithromycin, and spectinomycin. Further research is needed to correlate these promising *in vitro* results with clinical outcomes. A recent study evaluated the efficacy of *in vitro* interactions of 21 dual therapy combinations against 95 *N. gonorrhoeae* strains. Five of the 21 combinations were new introductions not covered by current standards and all five new combinations showed high synergistic effects on the strains tested. This suggests that additional *in vivo* evaluations in clinical trials are needed before these combinations can be used in the treatment of gonorrhoeae in the future. The combination of gentamicin and azithromycin is already recommended by various guidelines as an additional treatment option in the event of a failed first-line treatment option. A recent study investigated the synergistic effect of this combination with gentamicin in combination with five other antibiotics (cefixime, ceftriaxone, spectinomycin, azithromycin, moxifloxacin, and ertapenem). The study concluded that gentamicin, in combination with ertapenem or cefixime, has the greatest efficacy and synergistic effect on 75 strains of *N. gonorrhoeae* and can be offered as a new dual antibacterial treatment.

Newer antimicrobial agents for treatment of *N. Gonorrhoeae*

Zoliflodacin

Zoliflodacin targets the GyrB subunit of DNA gyrase (DNA gyrase subunit B) and re-stabilizes the cleaved covalent complex of DNA gyrase and inhibiting double-stranded DNA to generate circular DNA. Zolifloxacin and ciprofloxacin have a similar mechanism of action, but zolifloxacin does not use Mg^{2+} ions like ciprofloxacin. Studies have shown that zolifloxacin is also effective in ciprofloxacin-resistant strains. In general, no cross-resistance with previously discovered antibacterial agents has been observed, and zoliflodacin is suitable for gonococcal strains that are resistant to the antibiotics currently in use. Although no strains resistant to zoliflodacin have been identified so far, *in vitro* studies have shown that the amino acid substitutions D429N and K450N/T of GyrB play an important role in increasing MICs. *In vitro*, Zoliflodacin rapidly inhibited the development of *N. gonorrhoeae* and showed significant *in vitro* effects on geographically, chronologically and genetically diverse strains, including strains resistant to ceftriaxone. With the exception of ceftriaxone, where all values were essentially equivalent, zoliflodacin showed lower modal MICs, MIC50's, and MIC90's than all previously used antibiotics. The treatment success rates for genitourinary gonorrhoeae were 98% and 100% for zoliflodacin doses 2 g and 3 g, respectively, 100% for rectal gonorrhoeae, and 67%-78% for pharyngeal gonorrhoeae. Although the treatment success rate for pharyngeal gonorrhoeae is low, the number of patients in this group is small, with 6 and

9 patients receiving zoliflodacin 2 g and 3 g, respectively. In these cases, zoliflodacin was less effective than ceftriaxone. With the exception of pharyngeal gonorrhoeae, zoliflodacin appears to be effective and well tolerated, with most of the side effects being transient gastrointestinal disorders. Zoliflodacin is currently in Phase III clinical trials. Meaning, this study is still enrolling people and has not yet published its results.

Solithromycin

Solithromycin is a fourth-generation broad-spectrum macrolide that targets ribosomal bacterial sites and has been extensively studied in recent years. *In vitro* experiments with 246 *N. gonorrhoeae* isolates containing the multidrug-resistant strains H041 and F89 showed better antibacterial activity than the antibiotics currently in use (including ceftriaxone and azithromycin). Next, a phase 2 clinical trial was conducted using two oral doses of solithromycin, 1200 mg and 1000 mg. The efficacy of both doses in a total of 59 people was determined at 100% at all three sites (genital, oral, rectal). The only side effect was a mild dose-related gastrointestinal problem that did not interfere with treatment. Despite these results, a phase 3 clinical trial comparing solithromycin (1000 mg) with double treatment of ceftriaxone and azithromycin in uncomplicated genital gonorrhoeae failed to demonstrate non-inferiority. In addition, the incidence of adverse events was higher in the solithromycin group (53% *vs*. 34%), with diarrhoea (24% vs. 15%) and nausea being the most common (21% *vs.* 11%). Despite these disappointing results, it is unclear whether the discrepancies in results may have been corrected by dose changes and whether the failure was due to reinfection. In addition, the eradication rate difference of only 4% between groups can be explained by 8% of solithromycin patients with HIV-related immunosuppression.

Gepotidacin

Gepotidacin is a new antibiotic in the triazaacenaphthylene family that inhibits bacterial DNA gyrase and topoisomerase IV by a different mechanism than previous antibiotics, including quinolones. *In vitro* experiments showed that gepotidacin has potent activity against all *N. gonorrhoeae* strains, including multidrug resistant strains with MIC. The ParC D86 mutation associated with fluoroquinolone resistance was associated with an increase in gepotidacin MIC, but was not cross-resistant to other antibiotics. A phase II clinical trial testing a single dose of gepotidacin at a dose of 1500 or 3000 mg for the treatment of uncomplicated genitourinary gonorrhoeae has 96% (66/69) cure in the genitourinary, pharyngeal and rectal sites. Three failures were quinolone resistance due to ParC D86 substitution, one for rectal infection and the other two for genitourinary infection. Diarrhoea (27%), gas (23%), and stomach upset (15%) were the most common non treatment-limiting side effects. Based on these promising results, Gepotidacin will conduct a Phase III clinical trial comparing oral Gepotidacin with intramuscular ceftriaxone and azithromycin dual therapy for the treatment of uncomplicated genitourinary gonococcal infections.

Vaccine development for *N. Gonorrhoeae*

As *N. gonorrhoeae* develop resistance to antimicrobial treatments, the development of a vaccine becomes increasingly important. Mathematical modelling shows that even with a vaccine with 7.5 years of protection and 100% efficacy, or a vaccine with durable protection and 50% efficacy, gonococcal infections can decrease by up to 90% after 20 years when given in early adolescence. However, previous vaccination attempts have mostly been unsuccessful. In clinical trials in the 1970's, crude inoculation with dead whole cells proved successful in generating an antibody response but failed to elicit an adaptive immune response. Another vaccine using the gonococcal pilin failed in a large field trial and heterologous challenge research, which was attributed to the antigenic diversity of pilis. A third failed attempt focused on the protein porin, which was also attributed to genetic variance. In general, vaccine development is hampered by a lack of naturally acquired immunity against *N. gonorrhoeae* due to its high antigenic diversity, diverse immune evasion mechanisms, and host confinement to humans. However, increasing elucidation of *N. gonorrhoeae's* methods of evading the immune system, humanized mouse models, and identification of interesting targets have reignited attempts at vaccine development.

Current efforts and targets for *N. Gonorrhoeae* vaccine discovery

A previous study used the MeNZB vaccine against *N. meningitidis* in a case-control study in New Zealand to provide the first report of potential protective immunity against gonorrhoeae. This study examined reported cases of gonorrhoeae in New Zealand from 2004 to 2016 and found that those who had the MenNZB immunization had lower rates of infection, with an estimated vaccine efficacy of 31%. In a second retrospective cohort study, MeNZB to be 24% effective against hospitalizations due to gonococcal infections, validating the vaccines' cross-protection were identified. While effectiveness was moderate, dropping to 9% after 5 years, durability is less of a concern as the risk of gonorrhoeae infection decreases dramatically after age 30, making ongoing protection unnecessary. Other studies have also found that vaccination against meningococcal serogroup B can protect against gonorrhoeae. Analysis of gonorrhoeae rates in Cuba and Norway both showed a decrease in incidence following MenB vaccination efforts. The MeNZB vaccine is no longer available, but Bexsero, a newer serogroup B vaccine, contains the same Outer Membrane Vesicle (OMV) components as MeNZB, plus three recombinant proteins, one of which is Neisserial Heparin Binding Antigen (NHBA), a target, which has been shown to be important for gonococcal colonization and survival, which is conserved and expressed on the surface of *N. gonorrhoeae*. The vaccine not only effectively demonstrated anti-gonococcal antibodies caused by the OMV's, but also produced anti-gonococcal NHBA antibodies, providing an additional source of protection against the bacteria. These results were supported by studies in mice evaluating cross-protection of the vaccines, which showed faster clearance rates and lower *N. gonorrhoeae* burden with antibodies recognizing numerous *N. gonorrhoeae* surface proteins, including NHBA. Bexsero is currently in Phase II clinical trials (NCT04350138) with completion expected in August 2023. Efforts to produce a whole cell-based vaccine against *N. gonorrhoeae* have also been renewed. In another study, the researchers developed a transdermal whole-cell based inactivated gonococcal micro particle vaccine formulation. The proposed advantages over previous vaccines and other whole-

cell preparations are, first, the use of formalin-fixed whole gonococci, which protect all immunogenic epitopes from degradation, and second, the use of microparticles, which mimic the shape of the *N. gonorrhoeae cocci* shape, thereby activating the immune system, without suppressing it, and finally, transdermal delivery using microneedles, which allows for a slow, sustained release of antigens. To date, the vaccine has only been tested in mouse models *in vitro* and *in vivo*, where a significant increase in antigen-specific IgG titres has been detected. Further research is needed to determine if this vaccine can provide immunity against the isogenic vaccine strain as well as cross-protection against different *N. gonorrhoeae* strains. In addition, efforts are being made to develop alternative methods of antigen presentation for vaccination. In a previous study, Salmonella enteritidis bacterial ghosts, which are empty bacterial cell envelopes, were used to deliver a *N. gonorrhoeae* DNA vaccine. Bacterial ghosts offered good DNA loading capacity upon delivery to both professional and non-professional APCs, resulting in elevated levels of *N. gonorrhoeae* PorB-specific serum antibodies in mice compared to animals without ghosts. Some other work is being done in developing a vaccine using Helicobacter pylori ferritin nanoparticles to deliver *N. gonorrhoeae* antigens. Another study successfully demonstrated this presentation system with influenza and Epstein-Barr viruses, resulting in enhanced immune responses and protection against the viruses. Aside from the OMV's and NHBAs mentioned above, there are several other potential vaccine targets. In general, a target that is highly conserved across all *N. gonorrhoeae* strains would be excellent, first, some of the proteins used by *N. gonorrhoeae* for complement evasion, feeding, protein synthesis machinery, lysozyme inactivation, and host-glycan interaction have been suggested as possible vaccine targets. BamA, a protein from the aforementioned-barrel outer membrane complex, has been demonstrated was shown to be ubiquitously produced under varied different growth circumstances conditions and to elicit antibodies that cross-reacted with multiple distinct several different *N. gonorrhoeae* strains. Further research on these targets should be emphasized in order to enable the development of both treatments and vaccines, regardless, there are a number of additional other intriguing vaccine targets. The For example, the Lipooligosaccharide (LOS)-derived (LOS) derived epitope 2C7, for example, 2C7 is another target of interest. Although the LOS varies greatly depending on the phase, 2C7 is a widely expressed widespread virulence factor that has been proven to be crucial for gonococcal colonization shown in the experimental context. A recent research study identified cross-reactive IgG antibodies with complement-dependent bactericidal activity in mice inoculated vaccinated with a peptide mimic peptidomimetic of 2C7, resulting in quicker more rapid clearance of vaginal colonization and decreased reduced gonococcal loads. A publication reported on the generation of a tetrapeptide derivative based on this prototype peptide mimic vaccination in order vaccine to create a homogenous homogeneous and stable vaccine candidate TMCP2. When tested in mice with two separate *N. gonorrhoeae* strains, TMCP2 produced bactericidal IgG with less colonization and faster clearance, making TMCP2 a potential step toward an effective N. gonorrhoeae vaccine. As new attempts for a *N. gonorrhoeae* vaccine are revived, the recent elucidation of these new targets and biological survival mechanisms of *N.* gonorrhoeae promises successful vaccination.

Novel whole-cell-inactivated micro particles of *Neisseria gonorrhoeae* as a vaccine formula in micro needle-based transdermal immunization

The novel nanovaccine, encapsulated with whole-cell-inactivated *N. gonorrhoeae* and embedded in an albumin-based particle matrix, has the following advantages: a whole-cell-based vaccine covering all immunogenic epitopes; self-adjuvanted vaccine formulations enhance immunogenicity through the incorporation of outer membrane proteins and compounds such as endotoxin that are ligands to the Toll-Like Receptor (TLR), increased immune cell absorption and gradual antigen release (antigen depot effect); promoting strong autophagy formation that improves antigen presentation; heat-stable formulation that does not require refrigerated administration through a micro needle skin patch. A new gonorrhoeae vaccine was developed, consisting of biodegradable whole-cell formalin-fixed *N. gonorrhoeae* antigen encapsulated in microparticles and subsequently put in a micro needle "skin patch" enabling transdermal vaccine administration. *In vitro* cell-based experiments and an *in vivo* vaccination pilot research utilizing mice were used to investigate the new gonorrhoeae nanovaccine activity. The findings indicate that might be a potentially useful vaccination that generated antigen-specific antibodies as well as antigen-specific CD4 and CD8 T cell responses. More research is being conducted to define and establish this new gonorrhoeae nano vaccine using adjuvants, as well as to investigate the correlates of protection in inoculated mice.

Bioinformatic assessments of the variability of *Neisseria gonorrhoeae* vaccine candidates

With the increasing antibiotic resistance, the only plausible alternative to prevent the spread of gonorrhoeae is vaccination, however, the notorious phase and antigenic diversity of Neisseria gonorrhoeae surface proteins remains one of the hurdles in vaccine development. To support vaccine development efforts, extensive bioinformatics analyses of sequence variation by comparing 34 potential gonorrhoeae antigens among over 5,000 clinical isolates of N. gonorrhoeae was performed. Eight protein antigens showed exceptional conservation, with a single allelic variation found in more than 80% of the isolates and another 18 vaccine candidates were represented by three alleles in more than 50% of N. gonorrhoeae isolates from around the world. Phylogenetic analyses showed that AniA and FetB were the most closely related antigenic variants and that AniA and FetB were the most closely related antigenic variants between *N. gonorrhoeae* and *N. meningitidis*. Premature stop codons are found in up to 44% of N. meningitidis alleles for both antigens, indicating differential expression. Low-frequency surface polymorphisms were discovered by mapping polymorphisms onto the existing three-dimensional structures of 12 antigens. PorB and TbpB both had a significant proportion of polymorphism sites, TbpA was similarly highly diverse, however, conserved loops were discovered and the distribution of a single antigen variant among N. gonorrhoeae strains worldwide or low-frequency sequence polymorphisms in surface loops. Finally, the regularly used strain N. gonorrhoeae FA1090 proves to be a prototype vaccine, as it contains antigen sequence types identical to the most common antigenic variations.

Proposed human vaccine model

A higher level of complement-dependent bactericidal antibody activity directed against *N. gonorrhoeae* has been postulated as a predictor of protection against gonococcal infection and thus as a proxy for vaccination efficacy. Bactericidal antibodies, in addition to directly killing gonococci, increase phagocyte binding and uptake via Fc and complement receptors, which can kill infected organisms, however, a variety of factors affect the potency of bactericidal antibodies. In the context of natural infection, the major antigenic targets of bactericidal antibodies are poor and LOS components of the gonococcal outer membrane, which can vary both antigenic and, in their propensity, to produce bactericidal antibodies during infection. In addition, the presence of blocking antibodies directed against Rmp reduces the effective bactericidal effect of antibodies directed against these antigens. The soluble complement regulators C4BP and FH, also inhibit the bactericidal function of antibodies. Regulation by any cause can be overcome by sufficiently high quantities of bactericidal antibodies, a state unusual in natural infections but possible with a right vaccination approach. A number of gonococcal surface components that elicit bactericidal antibodies have been identified and are being pursued as vaccine candidates. Some of these targets do not promote bactericidal antibody activity in natural infection and have not been predicted as potential candidates for immune responses occurring during infection. They are examples of where nutrition trumps nature because an immune response can be forced under vaccination conditions that do not occur with natural infection. In addition, bactericidal antibody responses to various antigens can target essential physiological activities that, if compromised, could further limit *N. gonorrhoeae*, including colonization and invasion, feeding, and immune evasion. Proteomic analysis of *N. gonorrhoeae* surface proteins and bioinformatics analysis of an Adhesion Complex Protein (ACP) homologue originally found in *N. meningitidis* in *N. gonorrhoeae* identified additional vaccine candidates that elicit bactericidal antibodies. Other vaccine options that target function but have not been shown to produce a bactericidal effect are also examined in two review articles authored by us and colleagues.

The *Neisseria gonorrhoeae* Methionine sulfoxide reductase (Msra/B)

It was evaluated as a viable vaccine candidate in terms of expression, sequence conservation, localization, immunogenicit y, and functional activity of antibodies raised against *N. gonorrhoeae* Methionine sulfoxide reductase (MsrA/B). Gonococci MsrA/B has previously been shown to convert Methionine sulfoxide (Met (O)) in damaged proteins to Methionine (Met) and protect against oxidative stress. It was observed that the MsrA/B gene is present, highly conserved and expressed in all *N. gonorrhoeae* strains tested and that MsrA/B is surface exposed on *N. gonorrhoeae*. Recombinant MsrA/B is immunogenic, and mice vaccinated with MsrA/B plus either aluminum hydroxide gel adjuvant or Freund's adjuvant elicited a humoral immune response that was dominated by IgG1 antibodies. IgG2a, IgG2b and IgG3 titres were higher in mice vaccinated with MsrA/B Freud's adjuvant than in animals immunized with MsrA/B aluminum hydroxide adjuvant, while IgM titres were comparable for both adjuvants. MsrA/B-Freunds-derived antibodies induced bacterial death in mice *via* both serum bactericidal activity and opsonophagocytic activity. Anti-MsrA/B was also able to functionally quench MsrA/B activity by preventing binding to its substrate Met (O). It is believed that recombinant MsrA/B is a potential *N. gonorrhoeae* vaccine antigen.

Outer membrane vesicle vaccines for *Neisseria gonorrhoeae*

Epidemiological research has demonstrated that vaccines licensed for use against the closely similar bacteria Neisseria meningitidis may have only a marginal effectiveness against *N. gonorrhoeae* infections. A New Zealand case-control study found that subjects vaccinated against meningococcal serogroup B with the OMV vaccine MeNZB had significantly less gonorrhoeae than unvaccinated controls, and MeNZB had a predicted vaccine efficacy of 31% against *N. gonorrhoeae*. 4CMenB, a more modern vaccination, comprises the MeNZB-OMV's as well as three recombinant protein antigens; Human antibodies produced against 4CMenB are cross-reactive with *N. gonorrhoeae*. These findings imply that protective antigens exist for *N. gonorrhoeae* and that a vaccine-induced immune response may provide protection. Several studies and clinical trials are focused on developing OMV vaccines, identifying key antigens in OMVs, understanding the immune response elicited by OMVs, and determining the extent of potential OMV-mediated protection against *N. gonorrhoeae*. The most recent study looked at vaccination with detergent-extracted OMV (dOMV) in a mouse model of lower genital tract infections. These Domv's were generated from N. meningitidis wild-type or mutant strains (OCh and ABR) expressing the major PorA, PorB, and RmpM Outer Membrane Proteins (OMPs) that are variable, immunodominant, and involved in evading the immune response involved. By day 7, animals vaccinated with OMP-deficient dOMVs but not wild-type dOMVs had a statistically significant increase in gonococcal clearance compared to control mice (56% OCh-, 77% ABR-immunized mice were eliminated *vs.* 17% alum-immunized, 19% unimmunized mice cleared). At day 10, all dOMV-immunized mice showed statistically significantly higher gonococcal clearance compared to control mice. In both studies, there was no reproducible, significant reduction in bacterial load in mice vaccinated with dOMV compared to animals immunized with alum, and sera from mice immunized with the dOMV vaccines produced no bactericidal activity against *N. gonorrhoeae*. In humans, antibody-dependent serum bactericidal activity is consistent with protection against *Neisseria meningitidis*; however, its relevance to protection against *Neisseria gonorrhoeae* is uncertain. It has been suggested that clearance in dOMV-immunized mice is related to serum and vaginal IgG antibodies cross-reacting with *N. gonorrhoeae*. Further research is needed to understand the relationship between antibody levels, functional activity and gonococcal clearance of particular interest is the discovery of PorA, PorB, and RmpM removal from ABR dOMVs resulted in the production of antibodies that recognized a wider variety of gonococcal antigens than dOMVs from the wild-type strain. Serum from vaccinated mice was also used to discover cross-reactive proteins and potential vaccine targets such as PilQ, MtrE, NlpD and GuaB. This work is a step toward developing a gonococcal vaccine, but also underscores the limitations and unpredictability of the mouse model of gonococcal infection.

DISCUSSION

Complementary omics technologies have been established over the past two decades to study Neisseria genomes and gene products. Proteomic approaches used for Neisseria whole, outer-membrane and outer-membrane vesicle vaccines have generated protein maps and allowed researchers to study the effects of environmental stress on protein expression. In particular, immunoproteomics has uncovered proteins whose expression corresponds to the development of humans' natural immunity to infection, colonization, and post-vaccination. Neisseria proteomics methods have generated a catalogue of possible vaccine antigens, and exploring the functional and biological aspects of these proteins may one day lead to universal Neisseria vaccines. However, a number of key obstacles to the effective development of gonorrhoeae vaccines have been identified. The fact that the process of infection and the nature of drug resistance for this virus are not well understood is a major obstacle. Similarly, the gonococcal mechanisms of host immune protection have not been fully understood. Another problem is that spontaneous *N. gonorrhoeae* infection does not develop immunity or protect against reinfection. Because *N. gonorrhoeae* has the potential to suppress and distract host immune responses, gonorrhoeae infections are associated with only a transient and weak mucosal immune response. The majority of vaccination advances are based on the premise of duplicating natural immunity to infection, as a result, traditional options for gonococcal immunization, such as using live attenuated or inactivated *N. gonorrhoeae*, are ineffective. Despite these constraints, the successful development of vaccines against human papillomavirus (HPV, the most common sexually transmitted infection), the development of vaccine technologies that use antigen engineering, efforts to optimize the immune response, and improved targeted vaccine delivery have led to optimism that vaccination against *N. gonorrhoeae* is possible. Most vaccine optimists, for example, believe that an effective gonococcal vaccine may be created using the protein-based meningococcal vaccine as a template; however, another barrier to vaccine development is the fact that various strains of infectious *N. gonorrhoeae* exhibit considerable variances in pathogenic activity. This heterogeneity suggests the likelihood of immune response variation based on strain and related virulence. Finally, the fact that *N. gonorrhoeae* is an obligatory human disease, leading to a lack of suitable test models for infection research, is probably the most relevant hurdle for clinical vaccine development. Due to the human-specific toxicity of *N. gonorrhoeae*, traditional animal models appear to be poor predictors at best, although animal models are still valuable, they need to be complemented by studies using human cells or clinical infections to produce meaningful results. To date, only female mice treated with 17β-estradiol have been consistently used as a small animal model for lower genital tract infections. Studies in this mouse model have shown that intraperitoneal administration of a Multiantigenic Peptide (MAP1) administered subcutaneously recombinant refolded porin, the of viral replication particles (rrPorB-VRP) and the nasally administered OMV vaccine. These research findings imply that effective vaccination must be multivariate to target multiple conserved epitopes. Keeping up with an evolving disease will require the development of new drugs and vaccines in the future. There are a limited number of potential therapeutic targets in *N. gonorrhoeae*, and the rate at which gonococci are developing resistance to current drugs is worrying. Developing novel therapies by focusing on the non-variable host cell structures that gonococci encounter during adherence, colonization, and invasion may be a viable method. The success of maraviroc, a retroviral anti-attachment drug that targets host immune cells, could serve as a model in this regard. Other treatment options such as immunomodulators and monoclonal antibodies that enhance host immunity could represent study avenues for drug discovery experts.

CONCLUSION

In this review, we observed the current status of antibiotic resistance in *N. gonorrhoeae* around the world, the relevance of modifying currently used antimicrobial therapies, and progress in the development of innovative drugs and vaccines. Antimicrobial resistance in *Neisseria gonorrhoeae* is increasing worldwide, although resistance and reduced susceptibility to ceftriaxone, the most commonly used first-line therapy, is uncommon, the finding of strains with significant resistance to ceftriaxone underscores the need for immediate action. More worldwide surveillance of AMR in *N. gonorrhoeae* is clearly needed and the development of molecular diagnostics to predict ceftriaxone resistance may improve the identification and treatment of these diseases. Approaches such as antibiotic repurposing could help minimize our reliance on ceftriaxone as very few antibiotic therapies exist for *Neisseria gonorrhoeae* and only a few promising options are in development, including zoliflodacin and gepotidacin, both of which are in phase III clinical trials. There are new molecules in the early stages of development, but they will likely take many years to reach clinical testing, if ever. In addition, attempts to produce vaccines using different routes of administration and based on highly conserved surface components of *N. gonorrhoeae* strains have recently been revived. Some of these targets have been highlighted and their preclinical efficacy in eliciting robust immune responses and efficient eradication of *N. gonorrhoeae* has been reported. Overall, great strides have been made in limiting the spread of *N. gonorrhoeae*, but much more needs to be done to adequately address *N. gonorrhoeae* as a global health problem.

LIMITATIONS

The current review's shortcomings include omitting non-English language publications. It is difficult to analyze the data representativeness since it is conceivable that many of the most third-world countries are not included in this review due to a lack of relevant data.

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Not applicable.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Jefferson A, Smith A, Fasinu PS, Thompson DK (2021). Sexually Transmitted Neisseria gonorrhoeae Infections Update on Drug Treatment and Vaccine Development. Medicines (Basel). 8: 11.
2. Semchenko EA, Seib KL (2016). Intractable problems require novel solutions: it’s time to get serious about

developing a gonorrhoea vaccine. Sex Transm Infect. 92: 561-562.
3. Unemo M, Shafer WM (2014). Antimicrobial Resistance in Neisseria gonorrhoeae in the 21st Century: Past, Evolution, and Future. Clin Microbiol Rev. 27: 587-613.
4. Russell MW, Jerse AE, Gray-Owen SD (2019). Progress Toward a Gonococcal Vaccine: The Way Forward. Front Immunol. 10: 2417.
5. León-Buitimea A, Garza-Cárdenas CR, Garza-Cervantes JA, Lerma-Escalera JA, Morones-Ramírez JR (2020). The Demand for New Antibiotics: Antimicrobial Peptides, Nanoparticles, and Combinatorial Therapies as Future Strategies in Antibacterial Agent Design. Front Microbiol. 24: 1669.
6. Unemo M, Lahra MM, Escher M, Eremin S, Cole MJ, Galarza P, Ndowa F, et al. (2021). WHO global antimicrobial resistance surveillance for Neisseria gonorrhoeae 2017–18: a retrospective observational study. Lancet Microbe. 2: 627–636.
7. Kirkcaldy RD, Weston E, Segurado AC, Hughes G (2019). Epidemiology of Gonorrhea: A Global Perspective. Sex Health. 16: 401-411.
8. Peters R, Maduna LD (2020). Drug-resistant gonorrhoea is a growing threat: A South African case study. Australia.
9. Chemaitelly H, Majed A, Abu-Hijleh F, Blondeel K, Matsaseng TC, Kiarie J, Toskin I, et al. (2021). Global epidemiology of Neisseria gonorrhoeae in infertile populations: systematic review, meta-analysis and metaregression. Sex Transm Infect. 97: 157–169.
10. Suay-García B, Pérez-Gracia MT (2018). Future Prospects for Neisseria gonorrhoeae Treatment. Antibiotics (Basel). 7: 49.
11. Młynarczyk-Bonikowska B, Majewska A, Malejczyk M, Młynarczyk G, Majewski S (2019). Multiresistant Neisseria gonorrhoeae: a new threat in second decade of the XXI century. Med Microbiol Immunol. 209: 95–108.
12. Unemo M, Shafer WM (2011). Antibiotic resistance in Neisseria gonorrhoeae: origin, evolution, and lessons learned for the future. Ann N Y Acad Sci. 1230: 19-28.
13. Maduna LD, Kock MM, van der Veer BMJW, Radebe O, McIntyre J, van Alphen LB, Peters RPH (2020). Antimicrobial Resistance of Neisseria gonorrhoeae Isolates from High-Risk Men in Johannesburg, South Africa. Antimicrob Agents Chemother. 64: 00906-00920.
14. Yan J, Xue J, Chen Y, Chen S, Wang Q, Zhang C, Wu S, et al. (2019). Increasing prevalence of Neisseria gonorrhoeae with decreased susceptibility to ceftriaxone and resistance to azithromycin in Hangzhou, China (2015–17). J Antimicrob Chemother. 74: 29–37.
15. Lin EY, Adamson PC, Klausner JD (2021). Epidemiology, Treatments, and Vaccine Development for Antimicrobial-Resistant Neisseria gonorrhoeae: Current Strategies and Future Directions. Drug. 81:1153-1169.
16. Vekemans J, Hasso-Agopsowicz M, Kang G, Hausdorff WP, Fiore A, Tayler E, Klemm EJ, et al. (2021). Leveraging Vaccines to Reduce Antibiotic Use and Prevent Antimicrobial Resistance: A World Health Organization Action Framework. Clin Infect Dis. 73: 1011–1017.
17. Gottlieb SL, Johnston C (2017). Future prospects for new vaccines against sexually transmitted infections. Curr Opin Infect Dis. 30: 77–86.
18. Gottlieb SL, Ndowa F, Hook EW, Deal C, Bachmann L, Abu-Raddad L, Chen XS, et al. (2019). Gonococcal vaccines: Public health value and preferred product characteristics; report of a WHO global stakeholder consultation, January 2019. Vaccine. 38: 4362-4373.
19. Connolly KL, Pilligua-Lucas M, Gomez C, Costenoble-Caherty AC, Soc A, Underwood K, Acintyre AN, et al. (2021). Preclinical Testing of Vaccines and Therapeutics for Gonorrhea in Female Mouse Models of Lower and Upper Reproductive Tract Infection. J Infect Dis. 224: 152–160.