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Review

Chlamydial plasmids and bacteriophages

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Chlamydia are absolute pathogens of humans and animals; despite being rather well recognised, they are still open for discovery. One such discovery is the occurrence of extrachromosomal carriers of genetic information. In prokaryotes, such carriers include plasmids and bacteriophages, which are present only among some *Chlamydia* species. Plasmids were found exclusively in *Chlamydia (C.) trachomatis, C. psittaci, C. pneumoniae, C. suis, C. felis, C. muridarum* and *C. caviae*. In prokaryotic organisms, plasmids usually code for genes that facilitate survival of the bacteria in the environment (although they are not essential). In chlamydia, their role has not been definitely recognised, apart from the fact that they participate in the synthesis of glycogen and encode proteins responsible for their virulence. Furthermore, in *C. suis* it was evidenced that the plasmid is integrated in a genomic island and contains the tetracycline-resistance gene. Bacteriophages specific for chlamydia (chlamydiaphages) were detected only in six species: *C. psittaci, C. abortus, C. felis, C. caviae C. pecorum* and *C. pneumoniae*. These chlamydiaphages cause inhibition of the developmental cycle, and delay transformation of reticulate bodies (RBs) into elementary bodies (EBs), thus reducing the possibility of infecting other cells in time. Plasmids and bacteriophages can be used in the diagnostics of chlamydioses; although especially in the case of plasmids, they are already used for detection of chlamydial infections. In addition, bacteriophages could be used as therapeutic agents to replace antibiotics, potentially addressing the problem of increasing antibiotic-resistance among chlamydia.

Keywords: Chlamydia, plasmid, bacteriophage

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INTRODUCTION

Chlamydia are absolute intracellular pathogens in humans, as well as vertebrates and invertebrates, all present in the environment, including aquatic environments (Pawlikowska & Deptula, 2012). Their virulence is principally due to components of the outer membrane (Inc and Pmp proteins, related to the third type secretion system) and various secretory proteins (e.g. glycosyltransferase), chromosomally encoded (Rockey *et al.*, 2000; Nunes *et al.*, 2013), and their virulence is supplemented with extrachromosomal factors, specifically plasmids (Song *et al.*, 2013, 2014). Chlamydia are distinct prokaryotes because they carry features that are intermediate between bacteria and viruses. The most characteristic feature that differentiates them from bacteria is their unique development cycle, lasting 48–72 hours, with three forms that include an infectious elementary body (EB), a non-infectious reticulate body (RB), as well as intermediate body (IB) (Horn, 2012; Pawlikowska & Deptula, 2012). Their genetic information, as in all prokaryotes, is written in a rather small bacterial chromosome (approx. 1 Mbp) (Nunes *et al.*, 2013), but some of their genotypic and phenotypic features are supplemented with extrachromosomal carriers, that is plasmids (Song *et al.,* 2013, 2014) and bacteriophages (Hsia *et al.,* 2000).

Plasmids are small, usually circular DNA particles which coexist in bacterial cells, carrying genetic information apart from the bacterial chromosome, and they are capable of independent replication (Petersen, 2011). They can transfer genes that are not usually present in the bacterial chromosome, and despite not being essential for bacterial cell functioning, they can condition features necessary for their survival (Petersen, 2011). Among others, plasmids carry genes responsible for resistance to antibiotics (resistance plasmids), genes encoding toxins, e.g. bacteriocins, genes conditioning degradation of chemical compounds (catabolic plasmids), genes conditioning virulence, and conditioning one of the variation mechanisms in prokaryotes — conjugation (conjugative plasmids). An important feature of plasmids present among prokaryotes, including chlamydia, is the fact that they can be transferred among bacterial cells, including horizontal gene transfer (Petersen, 2011). This feature of plasmids is a useful biotechnological tool used in genetic engineering (Petersen, 2011). Due to the range, plasmids have been divided into plasmids with narrow host range (NHR), such as those that replicate and exist exclusively in the native host and closely related species, and plasmids with broad host range (BHR), characterised with capacity of colonizing many unrelated species (Petersen, 2011). Chlamydial plasmids actually colonise just some species of chlamydia that are pathogenic to humans and animals (Pickett *et al.,* 2007).

Bacteriophages (phages) — bacterial viruses, including chlamydia, are all-present in the environment, as they have been isolated from water, soil, but also desert sand, hot springs, and even from humans or animals (Clockie *et al.,* 2011). They are characterised by narrow host range, although there are also phages infecting various bacterial species. After infecting bacteria, the phages can undergo two basic lifecycles — lytic or lysogenic, which begin in the same way, that is by adsorption on a bacterial cell *via* specific receptors. Further phases of the lifecycle involve permeation into the bacterial cell, release of the genetic material and passage into an eclipse phase, namely taking

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Abbreviations: C, Chlamydia; EB, elementary body; RB, reticulate body; IB, intermediate body; Inc proteins, inclusion membrane proteins; Pmp, polymorphic membrane protein; ORF, open reading frames; NAAT, nucleic acid amplification test

control over the host's metabolism. The next stages are replication of the genetic material, synthesis of viral proteins, formation of new bacteriophage particles and their release (Clockie *et al.,* 2011). Phage genetic material can be either DNA or RNA, in a single- or double-stranded form, in circular or linear form (Clockie *et al.,* 2011; King *et al.,* 2012). The current systematic of bacteriophages differentiates 10 families and 1 genus (not assignated to a family), among which 8 families and as well as the genus not classified into any family are bacteriophages containing genetic material in the form of DNA, whereas just 2 families contain RNA (Krupovic *et al.,* 2011; King *et al.,* 2012). There is also a proposal of a new family of bacteriophages — *Sphaerolipoviridae*, containing dsDNA as genetic material (Pawlowski *et al.*, 2014). Chlamydial bacteriophages belonging to the *Gokushovirinae* group in the *Microviridae* family, contain a single-stranded circular DNA particle (ssDNA) and their virion is in the cubic form and does not have an envelope (King *et al.,* 2012; Roux *et al.*, 2012).

CHARACTERISTICS OF CHLAMYDIAL PLASMIDS

Chlamydial plasmids are present in seven species of chlamydia (*C. trachomatis, C. psittaci, C. pneumoniae, C. suis, C. felis, C. muridarum, C. caviae*) and their length is about 7.5 kbp, present in the quantity of 4 to 10 copies (Pickett *et al.*, 2007; Rockey, 2011). Their replication is strictly related to the replication cycle of those bacteria, especially with divisions of RB (Ferreira *et al.,* 2013). Chlamydial plasmids are not conjugative plasmids, they mostly do not code antibiotic-resistance and do not have capacity for integration, except for integrative plasmid revealed in *C. suis* (Dugan *et al.,* 2004). The main role of plasmids in chlamydia is their participation in accumulation of glycogen, which is an additional material necessary for divisions inside chlamydial inclusion, which is related to the correlation of plasmid genes with the gene of glycogen synthase *glgA,* located at the chlamydial chromosome (Carlson *et al.*, 2008; Wang *et al.*, 2011).

The first chlamydial plasmids were described in the most recognised chlamydia, that is *C. trachomatis* and *C. psittaci* (Lovett *et al.*, 1980), although both wild and clinical strains of those bacteria could be plasmidless (An *et al.*, 1992; Farencena *et al.*, 1997; Ripa & Nilsson, 2006; Russell *et al.*, 2011). In *C. trachomatis,* so far five plasmids have been identified: pCTA, pCTT1, pCHL1, pLGV440 and pLGV2, in *C. trachomatis* serotype A (Carlson *et al.*, 2005), *C. trachomatis* serotype B (Sriprakash & Macavoy, 1987), *C. trachomatis* serotype D (Comanducci *et al.*, 1990), *C. trachomatis* serotype L1 (Hatt *et al.*, 1988) and *C. trachomatis* serotype L2 (Comanducci *et al.*, 1988) respectively. Also, four plasmids have been described for avian strains *C. psittaci*, plasmid pCpA1 from *C. psittaci* strain N352 (Lusher *et al.*, 1989; Thomas *et al.*, 1997); pCpA2 from *C. psittaci* strain 6BC (Lusher *et al.*, 1989), pCpA3 from *C. psittaci* strain 360 (Lusher *et al.*, 1989) and pCpA4 from *C. psittaci* strain RA1/56 (Lusher *et al.*, 1989), respectively. Plasmids have also been described from *C. pneumoniae* — strain N16 (plasmid pCpnE1) (Pickett *et al.*, 2005); in *C. felis* strain Fe/C-56 (plasmids pCfe1 and pCfe2) (Azuma *et al.*, 2006; Lusher *et al.*, 1989) in *C. muridarum* strain Nigg (plasmid pMoPn) (Thomas *et al.*, 1997; Read *et al.*, 2000) and in *C. caviae* strain GPIC (plasmid pCpGP1) (Read *et al.*, 2003). Plasmid from *C. suis* should be considered separately due the fact that it is an integrative plasmid, occurring as a genomic island (IScs605) together with some insertion sequences in the bacterial chromosome that encodes genes of resistance to tetracycline (*tet*C) (Dugan *et al.,* 2004, 2007).

Chlamydial plasmids contain non-coding RNA (Abdelrahman *et al.*, 2011) and eight open reading frames (ORF1-8) (Table 1), whereas the functions of five of them have been thoroughly recognised (Li *et al.*, 2008; Ferreira *et al.*, 2013; Song *et al.*, 2013). While comparing the sequences of plasmids isolated from various species of *Chlamydia*, it seems that their genetic similarity would range from 69 to 99% (Thomas *et al.,* 1997). Whereas in the case of plasmids isolated from *C. trachomatis*, from the strains representing 15 serotypes, high homology of their sequence was found (only 1% of differences was recorded), and is considered to be the most conserved among chlamydial plasmids (Thomas *et al.*, 1997; Ferreira *et al.*, 2013), similarly as *C. felis* plasmids (Di Francesco *et al.*, 2010; Harley *et al.*, 2010). Such high homology and conservative nature of plasmids of *C. trachomatis* and *C. felis* may suggest that they have evolved in parallel with *C. trachomatis* and *C. felis* (Seth-Smith *et al.,* 2009). Among others, their homology and conservative nature is a result, of the fact that there is a 22 bp region located between ORF8 and ORF1, which occurs in four copies in the plasmid sequence (Thomas *et al.,* 1997). Similar repetitive sequences have been revealed in plasmids originating from avian strain *C. psittaci* (Hugall *et al.*, 1989; Lusher *et al.*, 1989), from *C. felis* and *C. cavie* (Lusher *et al.*, 1989), and they have also been identified in plasmids from *C. pneumoniae* strain N16 (equine biotype) and *C. muridarum* strain E58 (Hugall *et al.*, 1989; Everett *et al.*, 1999). In the case of plasmids from *C. trachomatis* strains, their conservative sequences differ as compared to plasmids of *C. psittaci* by substitution of two nucleotides (Thomas *et al.,* 1997). When analysing nucleotide sequences of open reading frames, it was evidenced that proteins encoded by ORF1 (*pgp7*) and ORF2 (*pgp8*) are homologues of, integrase and recombinase, respectively, and are in charge of regulation of plasmid replication, while ORF3 (*pgp1*) is a homologue of DnaB helicase and participates in unwinding of the double DNA strands during their replication. In turn, ORF4 encodes Pgp2 protein which, depending on chlamydial species, contains from 345 to 245 amino-acids, but its function has not yet been determined. One of the major frames is ORF5, which encodes 28kDa protein Pgp3, which can be a marker of chlamydial infections (Comanducci *et al.*, 1994; Li *et al.*, 2008), although its function for other chlamydial features remains unknown. ORF6 encodes Pgp4 protein that contains 101–102 amino-acids, while ORF7 and OEF8 encode proteins Pgp5 and Pgp6, respectively, which may be involved in plasmid replication (Thomas *et al.,* 1997). Gong *et al.* (2013) have shown by using a *C. trachomatis* plasmid with pORF deletion, that Pgp3, Pgp4, Pgp5 and Pgp7 are not required for maintenance, and also minimum 30 nucleotides were found in *pgp3* which encode a region required for *pgp4* expression. In *C. muridarum* plasmid with deleted pORFs a new function of Pgp5 was discovered, which negatively regulates some plasmid-dependent genes (Liu *et al.*, 2014). Despite the occurrence of plasmids in many chlamydial species (*C. trachomatis, C. psittaci, C. pneumoniae, C. suis, C. felis, C. muridarum, C. cavie*), their role in the development cycle of these bacteria has not yet been fully understood. It was evidenced that plasmids encode various proteins involved in the replication of the plasmid itself, but it was not determined whether they affect the interaction with the host's cell; it was only demonstrated that Pgp3 protein is secreted to the cytosol of cells infected by chlamydia (Li *et al.,* 2008). The spreading mechanism of this

Table 1. Functions of chlamydial plasmid encoded proteins (Comanducci *et al*., 1994; Thomas *et al*., 1997; Li *et al*., 2008; Gong *et al*., 2013; Song *et al*., 2013; 2014)

Open reading frame	Protein	Function
ORF1	Pgp7	homologue of integrase
ORF2	Pgp8	homologue of recombinase
ORF3	Pgp1	homologue of DnaB helicase
ORF4	Pgp2	function unknown
ORF5	Pgp3	marker of chlamydial infection
ORF6	Pgp4	function unknown
ORF7	Pgp5	involved in plasmids replication/negative regulation of some plasmid--dependent genes
ORF8	Pgp6	involved in plasmid replication

protein is similar, but not identical, to the distribution of chlamydial CPAF factor (Chlamydia Protease-like Activity Factor), genome-encoded protease secreted to cytosol of a cell infected with chlamydia (Li *et al.,* 2008). It was demonstrated that Pgp3 protein stimulates murine macrophages *in vitro* to release pro-inflammatory cytokines: MIP-2 (murine IL-2 homologue) and TNFα, and can act as a receptor for TLR4 (Li *et al.,* 2008). This protein can also be used in anti-chlamydial vaccines (Donati *et al.,* 2003). The role of plasmids in pathogenicity of *Chlamydia* is not yet fully understood, although it has been shown that the most important for plasmids are genes *pgp1* (ORF3), *pgp2* (ORF4), *pgp6* (ORF8) and *pgp8* (ORF2), while of slightly less importance are *pgp3* (ORF5), *pgp5* (ORF7) and *pgp7* (ORF1) (Song *et al*., 2013). However, *pgp7* and *pgp8* genes are very significant for chlamydial plasmids, as they show homology with the family of phage proteins (integrase/recombinase) (Song *et al.,* 2014). It was also shown that *pgp4* gene affects the ability to accumulate glycogen in chlamydial inclusions, and the Pgp4 protein is a transcriptional regulator of plasmid and chromosomal genes of chlamydia, responsible for their virulence (Song *et al.,* 2013, 2014). Also, no correlation was found between the quantity of plasmid copy in chlamydia and its pathogenicity (Last *et al.,* 2014). An experiment with plasmid-free *C. trachomaits* — serovar L2, transformed with a plasmid carrying a deletion of a plasmid CDS5 gene, used in mice with a urogenital infection, showed that inflammatory response in mice was enhanced (Ramsey *et al*., 2014). What this means is that the CDS5 plasmid plays a critical role in virulence and infectivity of *C. trachomatis* (Ramsey *et al*., 2014). This was confirmed in mice with a urogenital infection which were treated with plasmid-less serovar E of *C. trachomatis*, which showed that infectivity of this strain was reduced when compared to the wild strain of *C. trachomatis* carrying plasmid (Sigar *et al*., 2014).

Chlamydial plasmids, due to their low variability, have found practical application in diagnostics, principally as a target in DNA amplification in NAAT (nucleic acid amplification test) (Fredlund *et al*., 2004; Schachter *et al*., 2006). With the use of this method, a new strain of *C. trachomatis* — Sweden2 (serotype E), was described in 2006 (Ripa & Nilsson, 2006). The difference in this strain resulted from the deletion of a 377 bp long fragment from the plasmid DNA, and the impossibility of detecting this strain using standard NAAT (Ripa & Nilsson, 2006). Studies of the evolution of both, *C. trachomatis* strains and their plasmids indicated that both the chlamydial chromosome and the plasmid DNA evolved in parallel, and both depend on the biotype or serotype of chlamydia (Seth-Smith *et al.,* 2009). These results suggest that the presence of plasmids with deletion within urogenital strains may cause difficulty in diagnosis of those chlamydial infections, and consequently leads to latent infections, effects of which can be severe, such as infertility. Another application of chlamydial plasmids is an attempt to use them in anti-chlamydial vaccines. It is known that chlamydial strains which do not carry plasmids are less pathogenic, and sometimes do not activate an immunological response (O'Connell *et al.,* 2007). It was demonstrated that *C. muridarum*, deprived of plasmids both *in vivo* and *in vitro*, does not activate a TLR2-dependent immunological response (O'Connell *et al*., 2007); this was also confirmed in the study with *C. cavie* on Guinea pigs (Frazer *et al*., 2012). In turn, Miyairi *et al.* (2011) did not observe differences in TLR2 activation in the case of the *C. psittaci* strain 6BC with and without plasmids. In addition, this study has shown that infection with both forms of the *C. psittaci* strain 6BC renders the infection (Miyairi *et al.,* 2011). Those studies (Miyairi *et al.,* 2011), as well as another (Kari *et al.,* 2011), suggest that chlamydial strains deprived of plasmids can be applied for immunisation, which may provide protection against infection with chlamydial strains carrying plasmids or weaken the effects of such infections.

Table 2. Functions of chlamydiaphage ORFs and their encoded proteins (Liu *et al*., 2000; Sait *et al*., 2011; Roux *et al*., 2012)

Open reading frame	Protein	Function
ORF1	VP1	major capsid protein
ORF2	VP2	minor spike protein, DNA pilot protein
ORF3	VP3	scaffold protein
ORF8	J	involved in DNA packaging
ORF4	A	non-structural protein, involved in the synthesis of viral DNA
ORF5	C	non-structural protein, involved in packaging of DNA into the viral capsid

CHARACTERISTICS OF CHLAMYDIAL BACTERIOPHAGES (CHLAMYDIAPHAGES)

Chlamydial bacteriophages belong to the *Chlamydiamicrovirus* genus, which together with *Bdellomicrovirus* and *Spiromicrovirus* form the *Gokushovirinae* group, which belong with *Microvirus* to the aforementioned *Microviridae* family (King *et al.,* 2012; Roux *et al*., 2012). The first chlamydial phage, denoted as Chp1 bacteriophage (Chlamydiaphage 1), was described in 1982 in reticulate bodies (RB) and inclusions of *C. psittaci* isolated from ducks (Liu *et al.,* 2000). At present, five more chlamydiaphages are known, namely Chp2 (Chlamydiaphage 2), Chp3 (Chlamydiaphage 3), φCPG1 (Guinea pig chlamydiaphage), φCPAR39 (φCpn1; *Chlamydia pneumoniae* AR39 bacteriophage) and Chp4 (Chlamydiaphage 4), which primarily infect *C. psittaci*, *C. abortus*, *C. felis*, *C. cavie*, *C. pecorum* and *C. pneumoniae*. Viral genomes similar to chlamydiaphages were also found in the human gut, lung and feces (Roux *et al*., 2012). The mutual feature of these chlamydiaphages is the similar size of their genomes (4.5–4.8 kbp) and encoding of eight open reading frames (ORF1-8) (Table 2), arranged in the following order: VP1-VP2-VP3-ORF8-ORF4-ORF5, wherein ORF6 and ORF7 overlap ORF1 and ORF2, respectively (Sait *et al*., 2011; Roux *et al*., 2012). The most important are frames ORF1-3 and ORF8, which encode the major capsid proteins VP1 — a major capsid protein (ORF1), VP2 — a minor spike protein (ORF2), and VP3 — a scaffold protein (ORF3). The VP1 protein comprises two major areas that are exposed on the virion surface that likely interact with each other (Garner *et al.,* 2004). The first region is located at position 216-299 bp, and reminds IN5 loop, while another area, referred to as INS, is located between 462 and 467 bp. Protein encoded by ORF8 is similar to protein J of φX174 phage and is characterised with high degree of sequence conservation in Chp2, Chp3, CPG1 and CPAR39. Another common feature of chlamydiaphages is that their lifecycle is a lytic cycle, although the release of new particles from the bacterial cell is still mysterious. In the case of φX174 phage, a gene encoding protein E, a specific inhibitor of peptydoglycan synthesis (acting as penicillin) was detected in its genome, which results is cell lysis (Young, 1992; Bernhardt *et al*., 2000; Zheng *et al*., 2008). A homolog of this gene is also present in the chlamydial genome (Kalman *et al.,* 1999). The function of this gene does not only refer to the inclusion membrane where replication of chlamydia and phage occur, but also to the cellular membrane of the eukaryotic cell where chlamydia multiply (Hsia *et al.,* 2000).

Chlamydiaphage Chp1 was isolated, as mentioned above, from *C. psittaci* infected ducks. Its genome is comprised of 4877 bp, encodes 11 proteins, and the content of GC pairs is relatively low, as amounting to 36.6%. Open reading frames have an ATG start codon preceded with the region which could be an RBS (ribosome-binding site), which suggests that each of them may encode protein. Moreover, there are additional places for starting transcription, namely ORF2a, 2b, 4a, and 5a, which allow for encoding four additional proteins (Storey *et al.,* 1989a, 1989b). The organisation of ORFs is such that reading frames ORF 6 and 2, as well as ORF1 and 7 overlap, while ORF8 probably encodes a non-functional protein. The analysis of N-terminal amino acid sequences showed that structural proteins VP1, VP2 and VP3 are encoded by ORF1-3, while VP1 protein shows partial similarity with major structural protein of the F capsid of φX174 bacteriophage (a typical species of *Microvirus*), and the protein encoded by ORF4 reveals homology with protein A (Storey *et al*., 1989b). Chlamydiaphage Chp2 was identified in *C. abortus* and *C. psittaci* (Liu *et al*., 2000; Salim *et al*., 2008). The length of its genome amounts to 4567 bp and encodes eight open reading frames, of which ORF1-5, ORF7 and ORF8 are homologous to sequences of Chp1. Similarly as Chp1, the Chp2 phage has three structural proteins VP1, VP2 and VP3. However, capsid proteins show the greatest similarity to proteins of phage φSpV4 (a typical phage of *Spiromicrovirus*). Similarity was also noticed between the small J protein encoded by ORF8 of the Chp2 phage with a J binding protein of phage φX174 (Liu *et al.,* 2000). Chlamydiaphage Chp3 was isolated from elementary bodies of *C. pecorum* and, similarly as in previously described chlamydial phages, its genome has also 4544 bp and is organised into eight ORFs (Garner *et al.,* 2004). Chlamydiaphage Chp4 is a phage infecting *C. abortus,* and its genome is comprised of 4530 bp, and also features 8 ORFs. ORF 4 and ORF5 encode non-structural proteins similar to proteins A and C isolated from φX174 phage, involved in synthesis of phage DNA and packaged into capsids. Chlamydiaphage φCPG1 (Guinea pig chlamydiaphage) isolated from *C. psittaci* (currently *C. caviae*), with the genome sized 4529 bp, features eight open reading frames, where ORF1-3 encode structural proteins (VP1-3), while ORF4 and ORF5 encode proteins VP4 and VP5. The segment of the genome comprising protein VP4 and sequences above the region may contain signals important for replication and/or regulation of gene expression of the phage (Hsia *et al.,* 2000). The analysis of VP1 protein sequences showed high similarity between homologous sequences of Chp1, φCPG1 and φSpV4 (Hsia *et al*., 2000). The described chlamydiaphage φCPAR39, also known as φCpn1, was isolated from *C. pneumoniae* - strain AR39. This is the first and only chlamydial phage isolated from bacteria causing diseases in humans. Its genome is comprised of 4524 bp, and divided into eight reading frames (Karunkaran *et al*., 2002; Rupp *et al*., 2007; Hoestgaard-Jensen *et al*., 2011). In case of bacteriophages of *C. caviae* and *C. pneumonia,* three chlamydiaphage genes were found in genomes of their host (Read *et al*., 2003). This is similar to the *Microviridae*-related prophages integrated in the genome of *Bacteroidetes* and integration into the host's genome can establish lysogeny (Krupovic & Forteree, 2011).

The description presented indicates that all five chlamydiaphages (Chp1, Chp2, Chp3, Chp4, φCPG1, φCPAR39) have similar genome structure, although for the assessment of similarity or differences between them two regions present in all of them are used, namely the region encoding protein VP1 and ORF4 (Read *et al*., 2000; Everson *et al*., 2002; Sait *et al*., 2011). In the VP1 region, two characteristic regions are considered, that is IN5 and INS, closely located, and play a role in recognising the host's receptors (tissue tropism determination) (Everson *et al*., 2002). Sait *et al.* (2011) observed that adsorption of Chp2 or Chp3 phages on *C. abortus*, does not prevent adsorption of φCPAR39, which indicates and proves the presence of various receptors on the surface of chlamydia. It was also evidenced that replication initiation protein, encoded in the ORF4 region, is the fastest evolving protein as compared to other chlamydial proteins, and despite the fact that its function has not been entirely recognised, it was documented that its central sequence is characteristic of many prokaryotic Rep proteins (controlling replication) (Read *et al.,* 2000). The impact of bacteriophages on chlamydial development is still questioned, and thus the course and pace of infecting the macro-organism with them. In the studies on infection with Chp2 of *C. abortus*, inhibition of cellular division was observed in the form of blocking the transformation of RB into EB bodies, with simultaneous increase in the volume

of RB bodies (Salim *et al.,* 2008). It was also determined during the *C. abortus* cycle that between hour 36 and 48, increased replication of Chp2 phage genome occurs, coinciding with expression of phage proteins (VP3 — procapsid proteins) and replication of bacterial chromosome (Salim *et al.,* 2008). In the case of φCPG1 phage, it was evidenced that infection of *C. psitacii* — Guinea pig strain (presently *C. caviae*) occurs upon transformation of infectious EB bodies into metabolically active forms, namely RB. At that time, reticulate bodies RB grow (formation of maxiRB), which become the place of phage replication and maturation (Hsia *et al.,* 2000). Both, in the case of phage Chp2 infecting *C. abortus,* and φCPG1 infecting *C. caviae*, when new bacteriophage particles are formed, lysis of chlamydia as host's cell occurred. Chlamydial phages can occur in the cell's cytosol, but most frequently bind to the cellular membrane of EB bodies, and are transferred in such a form together with chlamydia to other eukaryotic cells. In case of mixed intranasal infection with *C. caviae* and stock of phage φCPG1, a positive reaction in serum of Guinea pigs — high level of antibody to VP1 was observed (Rank *et al*., 2009).

CONCLUSION

Plasmids and bacteriophages occurring in chlamydia show highly conservative nucleotide sequence, similarly as their hosts. Chlamydial plasmids generally do not carry for e.g. the genes of antibiotic resistance, as in other bacteria, except for plasmid *C. suis*, which is additionally an integrative plasmid, but carry the genes "cooperating" with chromosomal genes in accumulation of glycogen and encode proteins that may take part in virulence by interaction with chromosomal genes (Pgp4), and immunogenic proteins (Pgp3). They are used in diagnostics, DNA amplification tests, or as markers of chlamydial infection in the form of proteins encoded by them. They can be used as elements of vaccines preventing chlamydioses in animals. However, bacteriophages infecting chlamydia decrease their pathogenicity and distort their cycle by their replication. The easy transfer of infectious EB bodies into other cells can be used in the treatment for chlamydioses, particularly in the case of concealed and latent infections, principally at the time of chlamydial resistance to antibiotics used by choice of treatment (tetracyclines). Considering the presented and the still undiscovered properties of plasmids and chlamydiaphages, additional elements of genetic information present in chlamydia, it seems they will allow creating new tools for fighting chlamydioses in the future.

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