sequences makes it difficult to identify them although, they share some common characteristics: the presence of the said transmembrane domains; the highly charged and hydrophilic C-terminal domains; and the proximity of their encoding gene to that of the endolysin (Saier and Reddy 2015).

The function of these proteins is not yet well defined. Although it is clear that they confer access of endolysins to the bacterial peptidoglycan, it is not clear if that access is made through secretion, leakage, or membrane lysis, something that may depend on the holin type (Saier and Reddy 2015).

### 15.4.2 Holins as Antimicrobials

Besides their ancillary function in bacterial lysis, holins can cause cell death independent of endolysins and, unlike these, have an unspecific broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The holin HolGH15 from the *S. aureus* phage GH15 showed efficient antimicrobial activity on *S. aureus* and also on *L. monocytogenes*, *B. subtilis*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli*. The antimicrobial activity was most notorious on *Staphylococcus* and *Listeria*, with reductions close to 5 log units. Importantly, HolGH15 did not induce erythrocyte rupture on defibrinated rabbit blood, showing that this protein that exerts its function on biological membranes somehow unexpectedly does not cause hemolysis (Jiang et al. 2016).

A metagenomic study of a goat skin surface identified a holin-like protein shown to complement the holin function in a lysis-defective bacteriophage lambda. The 34-amino-acid protein named Tmp1 presented antimicrobial activity on a panel of Gram-positive bacteria, but not on Gram-negative bacteria. By using error-prone PCR, four mutants of Tmp1 were obtained with an increased spectrum of antibacterial activity that also includes Gram-negative bacteria, with reductions in the number of viable bacteria of up to 4 log units. The increased antibacterial activity seems to be related with an increased hydrophobicity (independent of the protein net charge), but still did not show hemolytic activity. This study, besides demonstrating the antimicrobial potential of holins, also shows that they can be improved through protein engineering (Rajesh et al. 2011).

Strangely, the *S. suis* bacteriophage holin HolSMP causes weak lysis of *S. aureus* and *B. subtilis* cells but not of the phage host (Shi et al. 2012). Also surprising, these two strains were insensitive to its cognate endolysin LySMP. The combination of these two phage lytic proteins, the holin HolSMP and the endolysin LySMP, extended the spectrum of both proteins in a synergistic effect since the combination presented antibacterial activity not only on strains that were sensitive to any of the lytic proteins but also on many other drug-resistant strains that were insensitive to each of the proteins alone (Shi et al. 2012). Such results demonstrate that the combined use of a holin and an endolysin presents a higher potential as antibacterials against drug-resistant strains.