Post-translational modification of ESKAPE pathogens as a potential target in drug discovery

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ESKAPE pathogens are gaining clinical importance owing to their high pervasiveness and increasing resistance to various antimicrobials. These bacteria have several post-translational modifications (PTMs) that destabilize or divert host cell pathways. Prevalent PTMs of ESKAPE pathogens include addition of chemical groups (acetylation, phosphorylation, methylation and hydroxylation) or complex molecules (AMPylation, ADP-ribosylation, glycosylation and isoprenylation), covalently linked small proteins [ubiquitylation, ubiquitin-like proteins (UBL) conjugation and small ubiquitin-like modifier (SUMO)] or modification of amino acid side-chains (eliminylation and deamidation). Therefore, the understanding of different bacterial PTMs and host proteins manipulated by these PTMs provides better insight into host–pathogen interaction and will also help to develop new antibacterial agents against ESKAPE pathogens.

Introduction

A group of pathogenic bacteria has emerged that can escape antibiotic treatment and demonstrates new concepts of transmission, pathogenesis, and resistance – collectively named ESKAPE pathogens by the Infectious Diseases Society of America (IDSA). This group includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species [1–4]. The widespread use, overuse, and misuse of antibiotics have all resulted in the emergence of these antimicrobial-resistant bacteria, posing a very serious threat to healthcare [5]. There are different approaches such as nanomedicine [6,7] or herbal [8–10] and *in silico* [11–15] methods that have been tried, but suitable therapeutics are not available for drug-resistant ESKAPE pathogens. Therefore, it is of immediate concern to find alternatives to the existing therapies to treat these diseases efficiently; and a better understanding of the factors involved in the virulence or pathogenicity, mechanisms of transmission, pathogenesis, morphological and behavioral changes during growth, the diverse survival strategy and adaptability to survive various stress conditions is essential. Therefore, the strategy to disarm the bacteria with new drug targets is a better alternative for reducing the development of quick resistance, preserving the gut microbiota and producing an increased repertoire of pharmacological targets [16]. Virulence factors such as adhesion, quorum sensing, secretion, and toxin production and oxidative folding are generally targeted for the development of new antivirulence agents [17–19]. Recently, the role of post-translation modification (PTM) in the virulence mechanism of bacteria has been investigated, which might serve as a potential target for the development of antibacterial or antivirulence agents.

More than 300 PTMs have been reported to date [20], including reversible or irreversible PTMs, attachment of chemical groups (acetylation, phosphorylation, methylation and hydroxylation) or complex molecules (AMPylation, ADP-ribosylation, glycosylation and isoprenylation), covalently linked small proteins [ubiquitylation and ubiquitin-like (UBL) protein conjugation] or the modification of amino acid side-chain residues (eliminylation and deamidation). The most common PTMs that are observed in bacteria are phosphorylation, acetylation, carboxylation, methylation, sumoylation, proteolysis, glycosylation, lipidation, nitrosylation, phosphoform addition and modification, adenylation, deamidation and ribosylation [21]. Bacteria also possess a large number of PTMs that destabilize or divert the host cell pathways, breaking the host defense and thereby promoting their replication [20,22]. The pathogens also alter the PTMs of their host to promote their own growth and survival in the host system. The modification of the host PTMs is generally brought about by different

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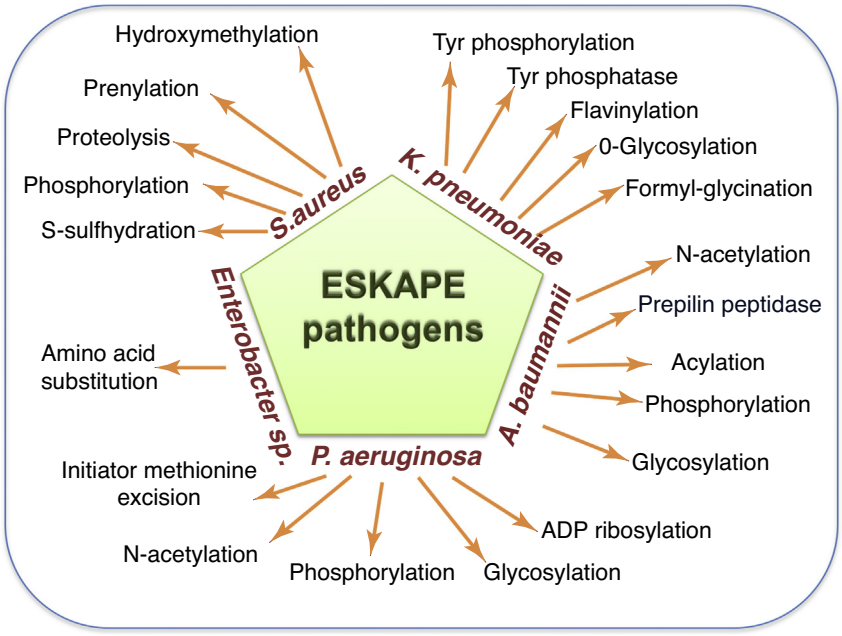

FIGURE 1

Schematic diagram showing different post-translational modifications (PTMs) identified in *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* sp.

bacterial effectors present at the surface of the bacteria or that are secreted and capable of interacting with the intracellular proteins or the plasma membrane of the host. The former is seen in the case of intracellular bacteria whereas the latter is with regard to extracellular bacteria that secrete toxins that can cross the host plasma membrane, or effectors directly introduced into the host cell by the bacteria via T3SS or T4SS (type III or IV secretion systems) [20]. Therefore, this review discusses different PTMs that ESKAPE pathogens exhibit (Fig. 1) or interrupt in the host cell to modulate the cellular functions of the host and subvert their defense system. Biological significances and functions of different PTMs found in the ESKAPE pathogens are listed in the table (Table 1).

PTM in ESKAPE pathogens

PTM in Staphylococcus aureus

S. aureus is known to express a vast range of virulence determinants, enabling the bacteria to survive host immune responses and cause various diseases. Prenylation is a crucial PTM that exists in *S. aureus* and involves the addition of a prenyl group either to CAAX motif in the C terminus of a protein or to a metabolite. Studies suggest that mutant strains that are not capable of undergoing prenylation exhibit smaller colony size, no pigmentation, growth defects as a result of low ATP levels and altered antibiotic resistance, increased sensitivity to antibiotics targeting the cell wall and elevated resistance to antimicrobial peptides (AMPs) and aminoglycosides. Hence, lack of prenylation changes the structure of the cell envelope by increasing the membrane fluidity and changing the fatty acid composition [23]. Another PTM (i.e, hydroxymethylation of asparagine and glutamine residues) of some proteins was observed when the bacteria were grown under specific conditions. The specific function of this PTM remains unknown but it can be associated with the modification of virulence factors owing to their presence on some surface proteins [24].

Apart from this, protein phosphorylation at cysteine residues also participates in the bacterial virulence. Using *S. aureus* infection in an *in vivo* mouse model, it has been shown that elevated protein Cys-phosphorylation significantly reduces staphylococcal virulence. Cysteine phosphorylation occurred in the staphylococcal accessory regulator A (SarA)/MarR family global transcriptional regulator A (MgrA) family of proteins and is mediated by the eukaryotic-like kinase–phosphatase pair Stk1–Stp1. Cell-wall-targeting antibiotics (e.g., vancomycin and ceftriaxone) inhibit the kinase activity of Stk1 and lead to decreased Cys-phosphorylation of SarA and MgrA [25]. This plays an essential part in changing the intracellular redox balance, hence leading to virulence and also contributing to antibiotic resistance [25]. PknB is the first proline-directed kinase with mitogen-activated protein kinase (MAPK)-like activity found in prokaryotes and is released by *S. aureus*. This enzyme phosphorylates bacterial protein and human host proteins; and its potential targets are concerned with immune responses, metabolism, apoptosis, and transport. Hence, secretion of PknB facilitates bacteria to escape intracellular killing and grow successfully [26]. Moreover, virulence of these bacteria is regulated by the Agr system (peptide-based quorum-sensing system) which includes an autoinducing peptide derived from pre-peptide AgrD, response regulator AgrA, receptor kinase AgrC and the protein AgrB – responsible for processing and PTM of AgrD. *S. aureus* also synthesizes some quorum-sensing molecules mediating virulence that are extensively modified to increase their stability and functionality. This group is classified as group I auto-inducing peptides (AIP) and is named group I cyclic thiolactone-peptide. The cyclic thiolactone linkage is crucial for specific self-activation of AIPs [27].

TABLE 1

Different post-translational modifications found in the ESKAPE pathogens and their biological significances

Bacteria	Post-translational modifications (PTMs)	Biological function	Refs
S. aureus	Prenylation	Cell growth and metabolism	[23]
	Intramembrane proteolysis	Activation of ECF sigma factor σS	[23]
	Hyxdroxymethylation of asparagine and glutamine residues	Modification of virulence factors	[24]
	Cys phosphorylation	Virulence and antibiotic resistance	[25]
	S-sulfhydration	Hydrogen sulfide signaling	[28]
	Post-translational cleavage	Ribosome assembly	[29]
	Protein carbonylation	Bacterial longevity	[30]
	Protein chlorination	Activated ribonuclease activity of YabJ	[85]
K. pneumoniae	Tyr phosphorylation	Capsular polysaccharides synthesis (K antigen)	[37,38]
		Phosphomannomutase (ManB)	[41]
		Undecaprenyl-phosphate glycosyltransferase (WcaJ)	[41]
		Protein-tyrosine kinase (Wzc)	[41]
		Yco6	[41–43]
	His phosphorylation	YeeO MATE channel	[50]
	Tyr phosphatases	Yco5 and Wzb	[41–43]
	Flavinylation	Alternative pyrimidine biosynthesis	[46]
	O-glycosylation	Synthesis of proline-rich antimicrobial peptides	[47]
	C-glycosylation	Synthesis of active microcin E492 protein toxin	[45]
	Cα-formylglycine	Catalytic residues in sulfatases	[44]
A. baumannii	Ser/Thr/Tyr phosphorylation	Metabolic regulation	[49]
	O-glycosylation	Biofilm formation and capsular polysaccharide synthesis	[52]
	N-acetylation	Cellular metabolic processes, biofilms formation, control phenotypic characters involved in stress adaptation, drug resistance and bacterial virulence	[54]
	Prepilin peptidase	Type IV pili synthesis	[98]
	Oligosaccharyltransferase	Biofilm formation and high virulence	[53]
	Glycosylation	Adhesion, protein stability, biofilm formation, flagellar filament assembly, pathogenicity	[51,99]
	Acylation	Colistin resistance	[57]
P. aeruginosa	ADP-ribosylation	Inhibit translation in the host	[69]
		Cellular adherence, enhance lamellipodia, maintain filopodia and ruffling of cells	[71]
	Ser/Thr phosphorylation	Destruction of the host defence system	[59]
	N-terminal acetylation	Unkown function	[61]
	Initiator methionine excision		
	Glycosylation	Twitching motility	[63] [66]
Enterobacter sp.	Gly substituted with any charged residue	Alteration of porin properties	[67]

Recently, it was seen that S-sulfhydration, a PTM in hydrogen sulfide (H_2S) signaling, was found in *S. aureus*. H_2S is thought to protect bacteria from oxidative stress and harbors significant effector molecules of H_2S signaling [i.e., reactive sulfur species (RSS), coenzyme A, etc.]. RSS levels modulate the expression of secreted virulence factors and are concurrent with S-sulfhydration-dependent inhibition of DNA binding by MgrA, a global virulence regulator. Similarly, thioredoxin-like proteins such as TrxP and TrxQ are also S-sulfhydrated in sulfide-stressed cells [28].

An N-terminal extension motif of L27 proteins immediately precedes the highly conserved A(S/H)KK motif. This extension is cleaved post-translationally in *S. aureus*, before or with ribosome assembly. Cysteine protease performs post-translational cleavage of L27. L27 and protease (termed Prp) are essential in *S. aureus*. Prp might provide an attractive target for the development of antibiotics specific to *S. aureus* and other Gram-positive pathogens in which this specific L27 processing occurs [29]. A carbonylated protein (as a marker of protein oxidation) is also seen in *S. aureus* and is associated with cell death involving protein ClpC (protein of the Hsp100/Clp ATPase family), thus contributing to bacterial longevity and chronic infection [30].

Bacterial defense against phages involved RNAi in plant and pattern-recognition systems (PRRs) in the vertebrates, recognizing nucleic acids and proteins of pathogen phages and activating the

interferon, proinflammatory and adaptive immune responses [31]. Serine/threonine kinases (STKs) play an important part at different stages of the antiviral response in eukaryotes, but eukaryotic-like STKs (eSTKs) have now been reported in most bacterial clades and involved in a variety of functions including cell-cycle control, exit of dormancy, cell wall synthesis, cell division, control of the central metabolism and virulence [32]. Recently, the *pgl* phage defense system from *Streptomyces coelicolor* [33] and related bacteriophage exclusion (BREX) systems of many unrelated bacteria [34] were described that are involved in bacterial defense systems. Prokaryotic CRISPR/Cas immune systems defend against phage infection using Cas nucleases and small RNA guides that specify one or more target sites for cleavage of the viral genome [35]. Stk2 is a staphylococcal STK that provides efficient immunity against bacteriophages by inducing abortive infection. A phage protein of unknown function activates the Stk2 kinase. This leads to the Stk2-dependent phosphorylation of several proteins involved in translation, global transcription control, cell-cycle control, stress response, DNA topology, DNA repair and central metabolism. Bacterial host cells die as a consequence of Stk2 activation, thereby preventing propagation of the phage to the rest of the bacterial population. This shows that mechanisms of viral defense that rely on protein phosphorylation constitute a conserved antiviral strategy across multiple domains of life [31].

PTM in Klebsiella pneumoniae

It has been reported that Ser/Thr/Tyr phosphorylation plays a crucial part in regulating the broad range of metabolic and physiological processes in prokaryotes [36]. In capsulated bacteria, Tyr phosphorylation mediates the synthesis of capsular polysaccharides (CPS; K antigen) which takes part in virulence [37,38]. The thick exopolysaccharide layer on their surface functions as the physical boundary to escape phagocytosis and, after that, complement-mediated killing further prevents activation of the complement system in the host [38–40]. Some of the proteins with phosphorylated tyrosine such as phosphomannomutase (ManB), undecaprenyl-phosphate glycosyltransferase (WcaJ) and protein-tyrosine kinase (Wzc) are located at the *cps* locus and are considered to be involved in the signaling cascades responsible for capsule production [41]. Yco6 is another Tyr kinase whereas Yco5 and Wzb are Tyr phosphatases that mediate capsule biosynthesis [41–43].

Recently, a PTM [i.e., Cα-formylglycine (FGly)] was found at the catalytic residue of sulfatases. FGly is generated by PTM of cysteine (prokaryotes and eukaryotes) or serine (prokaryotes) located in a conserved (C/S)*X*P*X*R motif. AtsB of *K. pneumoniae* is directly involved in FGly generation from serine and belongs to the radical *S*-adenosylmethionine (SAM) superfamily. *In vivo* and *in vitro* studies showed that SAM is the crucial co-factor for the formation of a functional AtsB-SAM-sulfatase complex, and FGly addition by AtsB [44].

Protein toxin microcin E492 (MccE492) is an 84-residue protein toxin that is produced and secreted by *K. pneumonaie* RYC492 and targets neighboring Gram-negative species. It is shown that PTM of MccE492 took place at the C terminus involving C-glycosylation and linearized enterobactin moiety catalyzed by MceCDIJ proteins. MceC and MceD catalyzed C-glycosylation at enterobactin, and MceI and MceJ attaches C-glycosylated enterobactins to the C terminus of MccE492 [45]. It was also shown in *K. pneumoniae* that ApbE is the only protein factor required for NqrC flavinylation and it was indicated that the reaction is magnesium-dependent and proceeds with FAD but not FMN [46]. Similarly, proline-rich antimicrobial peptides and AMP-like drosocin are synthesized in the hemolymph of *Drosophila melanogaster* upon bacterial (*Klebsiella*, *Salmonella*, etc.) challenge, bearing O-glycosylation as the PTM [47].

PTM in Acinetobacter baumannii

The first protein tyrosine kinase (Ptk) was discovered in 1996 in *Acinetobacter johnsonii* and categorized under the bacterial protein tyrosine kinase (BY kinase) family, which is used in the biosynthetic pathway of exopolysaccharides and their transport to form the matrix of the biofilm [36,48]. In *A. baumannii*, the percentage distribution of Ser/Thr/Tyr phosphorylation is dominated by serine (about 69%), threonine (24%) and tyrosine (5%) [49]. Recently discovered, histidine and aspartate phosphorylation has also been identified in *A. baumannii*. The phosphoproteomic analysis identified 31 His and 15 Asp sites in *A. baumannii* and 7 His sites in *K. pneumoniae*. The proteins involved are transcription factor AraC, zinc ion periplasmic transporter ZnuA, sulfate transport system permease CysW, twitching motility protein PilT, taurine-binding periplasmic protein TauA and inner membrane protein YfiN of *Acinetobacter baumannii* SK17. Similarly, the multidrug and toxic compound extrusion (MATE) channel YeeO in *K. pneumoniae* NTUH-K2044, branched amino acid transporter AzlC in *Vibrio vulnificus* and RNA-modifying pseudo-uridine synthase in *Helicobacter pylori* also use histidine and aspartate phosphorylation [50].

Glycosylation is responsible for bacterial adhesion, protein stability, biofilm formation, flagellar filament assembly and general virulence [51]. One of the PTMs, O-glycosylation, is observed in *A. baumannii*, which seems to be crucial for biofilm formation and capsular polysaccharide biosynthesis which is essential for evading the complement system [52,53]. The initiating glycosyltransferase, PglC regulates the biosynthesis of O-penta-saccharide-linked glycoproteins as well as the capsule, leading to the development of proper biofilm structures and virulence. Studies demonstrated that the penta-saccharides are not only attached to glycoproteins but also act as an integral part of capsule formation [52]. Investigations revealed that oligosaccharyltransferase (OTase) PglLAb transfers β-GlcNAc3NAcA4OAc-4-(β-GlcNAc-6-)-α-Gal-6-β-Glc-3-β-GalNAc, a penta-saccharide, to peptide regions rich in Ser, Pro, and Ala. PglLAb OTase mediates the formation of a well-defined biofilm and high virulence [53]. Moreover, repeating subunits of this penta-saccharide are prevalent in the capsule of *A. baumannii* ATCC 17978, suggesting a common pathway for capsule synthesis and O-linked protein glycosylation [52]. Reports also demonstrated that major pilin subunit, PilA, of the Tfp fiber undergoes glycosylation in the presence of pilin-specific oligosaccharyltransferase, TfpO, at the C-terminal serine. It was also reported that many *Acinetobacter* species express two functional oligosaccharyltransferases, one for pilin glycosylation and other for general protein glycosylation. Researchers have also discovered the ubiquitous O-linked protein glycosylation system within the genus *Acinetobacter*, which is OTase-dependent and has been found to participate in biofilm formation and pathogenicity of *A. baumannii* [53].

Recent studies revealed that N-acetylation plays a vital part in these bacteria. N-acetylation is not only required to maintain cellular metabolic process but also crucial for the formation of biofilms, controlling phenotypic characters involved in stress adaptation, drug resistance and bacterial virulence. N_α and N_ε are observed in *A. baumannii* [54]. Recently, ~525 proteins of *A. baumannii* were shown to have undergone N-acetylation (145 Nα-acetylation and 411 Nε-acetylation, 45 carry both these acetylations). N-acetylated protein plays an important part in bacterial virulence (iron acquisition), biofilm formation, stress adaptation and drug resistance of *A. baumannii* [54,55].

Type IV pili are crucial structures that play a part in *A. baumannii* virulence. The pilin subunit is the primary constituent of pilus fiber and assembly into Tfp fibers. Specific prepilin peptidase cleaves the N-terminal type III leader peptide sequence of the significant type-IV pilin subunits, which could undergo coupling with mono-methylation at the α-amino group of the new N-terminal phenylalanine [56]. Similarly, acylation, phosphorylation, and glycosylation of lipid A from *A. baumannii* confer resistance to colistin. Colistin-resistant strains of *A. baumannii* with alterations to the lipid A component of lipopolysaccharide (LPS) have been shown to be hepta-acylated with a phosphoethanolamine and galactosamine modifications [57].

PTM in Pseudomonas aeruginosa

ADP-ribosylation is one of the most studied PTMs catalyzed by many bacterial toxins. The ADP-ribose group is transferred from NAD by the toxins to Cys, Asn and Arg residues of many target proteins of the host to alter their functions, thereby making crucial metabolic processes. Numerous toxins are secreted by *P. aeruginosa*, resulting in the ADP-ribosylation of different target proteins. Experimental data suggest that exoenzymes S (ExoS), an ADP-ribosyl-transferase of *P. aeruginosa*, is translocated into eukaryotic cells and causes ADP-ribosylation of host Ras proteins and other GTP-binding proteins in the Ras superfamily, inhibiting target cell proliferation [58].

P. aeruginosa employs Hanks-type STK for phosphorylation of various targets that mediate the host–pathogen interaction to modulate different cellular metabolic and signal transduction pathways leading to bacterial adherence and invasion of the host system. However, the specific infection mechanism is not yet known but the mode of infection involves three stages: protein phosphorylation in the host cell, destruction of the host defense system by the kinase activity and, finally, the role of STKs by the unrevealed processes [59]. *P. aeruginosa* induces phosphorylation of the adaptor protein CrkII by activating the non-receptor tyrosine kinase Abl to promote its uptake into host cells. Host glycosphingolipid globotriaosylceramide (Gb3) is a signaling host receptor for *P. aeruginosa* lectin protein (LecA) that induces CrkII phosphorylation at tyrosine 221 [60].

Previous studies also suggest that N-terminal acetylation (NTA) and initiator methionine excision (NME) are two widespread modifications that have been observed in *P. aeruginosa*. Overall, 63% proteins undergo NME and 117 proteins undergo NTA and, in addition, specific proteins are recognized that are acetylated with initiator methionine [61].

Similarly, in *P. aeruginosa* strain 1244, the major pilin PilA is glycosylated by PilO (later renamed TfpO) – an O-OTase [62]. This was also found to mediate virulence because impaired twitching motility was observed in glycosylation-deficient mutants [63]. *P. aeruginosa* 5196 isolate (Pa5196) contains a group IV pilin (PilAIV) and is glycosylated via TfpW at multiple internal Thr and Ser residues by D-arabinofuranose [64,65]. It is also shown that lack of glycosylation of pilin in Pa5196 results in decreased abundance of PilAIV on the surface, which, in turn, reduces the ability to exhibit twitching motility [66].

PTM in Enterobacter *species*

Studies reveal that *Enterobacter aerogenes* are highly resistant to cephalosporins and show impaired uptake of the drug along with a decline in the sugar uptake and ion conductance. Modification in the amino acid sequence of porin from strain 3 (MOMP-3) by changing Gly with any charged side-chain residue such as Asn has resulted in altering the properties of the pore and thereby enhanced resistance toward cephalosporins [67]. Resistance toward multiple drugs can also be caused by the reduced level of porin expression in the outer membrane which can be attributed to overexpression of two outer membrane proteins: OmpX and LamB. Apart from this, the overproduction of OmpX and LamB causes modification of the LPS structure as well as expression of efflux pumps, which also contributes to the high level of resistance to different classes of antibiotics [68].

PTM in the host after interaction with ESKAPE pathogens and escape of ESKAPE pathogens from the host defense

Reports suggest that *P. aeruginosa* Exotoxin A targets the elongation factor 2 of the host, inhibiting the protein synthesis at the elongation step in a reticulocyte cell-free system [69]. This toxin exhibits a similar intracellular mechanism of action to that of the diphtheria toxin (DT) but differs in molecular and immunological properties, cellular specificities and the subsequent diseases caused [69]. Likewise, numerous virulence factors such as cytotoxins ExoY, ExoT, ExoU and ExoS are responsible for the pathogenicity of this bacteria and secreted by type-III secretary mechanism (T3SS) [70]. Among these, ExoT and ExoS are bifunctional with GTPase-activating protein activity at the C terminus, and ADP-ribosyltransferase activity at the N terminus. Experimental data suggest that ExoS of *P. aeruginosa* is translocated into eukaryotic cells by a bacterial type-III secretary mechanism activated by contact between bacteria and host cells. Inside the host cells, it causes ADP-ribosylation of Ras proteins and other GTP-binding proteins in the Ras superfamily, which inhibit target cell proliferation [58]. Proteins like ezrin, radixin, and moesin (collectively called ERMs) are also ribosylated by ExoS. The ERMs protein family is associated with the cytoskeleton of the cell, hence establishing the link between ADP-ribosylation and cytoskeletal changes and/or ultimately cell death. ADP-ribosylation of ERMs loses its capability to interact with their target proteins like Dbl or Rho and interfere with the Rho-mediated signal transduction pathway. Another effect of ADP-ribosylation is the prevention of ERMs to interact with F-actin, thus eliminating actin-related structures and playing an important part at cell–cell/matrix contact points, microvilli or membrane ruffles, thereby disrupting the cell adherence – one of the possible survival strategies of bacteria by the removal of the epithelial barrier. Moreover, some studies also reported that ADP-ribosylation activity of ExoS cytotoxin is associated with cellular adherence, enhancing lamellipodia, maintaining filopodia and ruffling of the cells [71]. ExoS ADP-

ribosylates the protein members of the GTPase Ras family, exhibiting different effects for different cell lines. The toxin could also target the ERMs in the immune cells where the ERMs take part in the association of antigen-presenting cells and T cells [72]. This will lead to down-regulation of the adaptive or innate immune response of the host [73,74]. One of the forms of ExoS, ExoS (ΔMLD), is not able to modify Ras *in vivo* very efficiently but induces actin rearrangements, which are cytotoxic [75]. CrkI/II proteins are ADP-ribosylated by ExoT [76], subsequently acting as an anti-internalization factor for this nosocomial pathogen [77]. Similarly, C3-like exoenzyme ADP ribosyltransferase produced by *S. aureus*, EDIN, acts on the GTP-binding proteins RhoA, B, and C, thereafter blocking their activation by guanine exchange factors. This will lead to significant changes or blockage of various Rho-mediating signaling pathways and, particularly, change in the actin polymerization [78,79]. Besides Rho GTPases, several bacterial toxins can also ADP-ribosylate actin, deregulating the host cell cytoskeleton.

Cif is a virulence factor secreted in outer-membrane vesicles by *P. aeruginosa* that increases the ubiquitination of ATP-binding cassette transporters like TAP1 and increases its lysosomal degradation in the proteasome of human pulmonary epithelial cells. This is achieved by reduced USP10 deubiquitinating activity, resulting in increased polyubiquitination and proteasomal degradation of TAP1. Reduction in TAP1 quantity decreases peptide translocation into the endoplasmic reticulum, which further reduces the availability of MHC class I molecules for presentation at the plasma membrane of human pulmonary epithelial cells and recognition by $CD8^+$ T cells [80].

P. aeruginosa infection also reduced CFTR expression and increased NHERF1 molecular weight through its hyperphosphorylation and ubiquitination as a consequence of bacterial pilin- and flagellin-mediated host cell interaction [81]. *P. aeruginosa* induces phosphorylation of the adaptor protein CrkII by activating the non-receptor tyrosine kinase Abl to promote its uptake into host cells. Host glycosphingolipid globotriaosylceramide (Gb3) is the signaling host receptor for *P. aeruginosa* lectin protein (LecA) that induces CrkII phosphorylation at tyrosine 221 [60]. The nucleotide oligomerization domain (NOD)-like receptor (NLR) family with caspase activation and recruitment domain (CARD) containing-3 (NLRC-3) protein has been implicated as a negative regulator of inflammation and antiviral and antibacterial responses. NLRC-3 promotes K48-linked polyubiquitination and degradation of interleukin (IL)-1 receptor-associated kinase-1. It was found that expression of NLRC3 was found to be decreased in mouse macrophages after *P. aeruginosa* infection and activate nuclear factor-κB (NF-κB) signaling and increase the production of proinflammatory cytokines [80].

Bacterial infection in the lungs induces an inflammatory response, which is a useful natural response to eliminate an invading pathogen; however persistent inflammation causes pneumonia. Alveolar macrophages and neutrophils (like phagocytes) kill internalized bacteria with alveolar macrophages. This is followed by subsequent infiltration of neutrophils in response to the chemotactic stimulus. Neutrophils generate noxious products including reactive oxygen species and proteases that are harmful to pathogens but also to the host. After clearance of a pathogen from the host, the immediate goal of the host is to mount an appropriate anti-inflammatory response to limit further neutrophil recruitment and to clear apoptotic neutrophils in the process of neutrophil efferocytosis. It is shown that lung $CD11b^+$ $Ly6G^{int}Ly6C^{lo}$ $F4/80^+$ cells, characterized as lung myeloid-derived suppressor cells (MDSCs), have an important function in neutrophil efferocytosis after infection with *K. pneumoniae*. A high concentration of phospholipid cardiolipin has been detected in the lung fluid of patients with persistent pneumonia. Cardiolipin is mainly confined in the inner mitochondrial membrane but, during cell death, it is released into the extracellular milieu as a mitochondrial damage-associated molecular pattern. Cardiolipin induces sumoylation of nuclear receptor peroxisome proliferator-activated receptor (PPAR)γ at K107, which causes recruitment of a repressive NCOR/HDAC3 complex to the IL-10 promoter, but not the tumor necrosis factor (TNF) promoter, hence inhibiting IL-10 production but not interfering with TNF production; thereby tipping the balance toward inflammation. This is the reason for persistent inflammation during pneumonia [81]. It was also recently seen that autophagy-related gene 7 (Atg7) downregulates NF-κB expression and proinflammatory cytokines during *K. pneumoniae* infection in macrophages (murine alveolar macrophage cell line, MH-S). On stimulation, IκB is phosphorylated, and phosphorylated IκB (p-IκBα) is subsequently ubiquitylated and degraded by the 26S proteasome, thus allowing NF-κB to translocate into the nucleus and initiate the expression of proinflammatory cytokine genes, such as TNF-α and IL-6. Loss of Atg7 shifts the binding of p-IκBα from Atg7 to ubiquitin, resulting in increased ubiquitylation of p-IκBα and intensified inflammatory responses against *K. pneumoniae*. This finding reveals a regulatory role of Atg7 in ubiquitylation of p-IκBα but also indicates potential therapeutic targets for *K. pneumoniae* control [82].

It has been seen that lysozyme present in the mucosal surfaces acts as muramidase that hydrolyses the peptidoglycan backbone of bacteria and produces the peptidoglycan fragments, which is recognized by the host receptors and initiates an immune response. To counterbalance, the pathogen modifies peptidoglycan residues surrounding the cleavage site to avoid the peptidoglycan degradation, hence blocking the immune response (proinflammatory fragments) [83]. These modifications include O-acetylation of MurNAc in Gram-positive bacteria (like *S. aureus*) and Gram-negative bacteria, N-glycosylation of MurNAc in *Mycobacterium tuberculosis*, O-acetylation of MurNAc Gram-negative bacteria like *Neisseria meningitidis*, N-deacetylation of GlcNAc in *S. pneumoniae*, among others.

The major families of host receptors that sense bacterial products include the Toll-like receptors (TLRs) and the Nod-like receptors (NLRs). TLRs predominantly sense the surface-associated host proteins, whereas NLRs sense cytoplasmic proteins. Peptidoglycan and its fragments are detected primarily via host receptors of the NLR family. Nucleotide-binding oligomerization domain-containing protein 1 (Nod1) is a host intracellular protein that is expressed in virtually all cell types. Nod1 senses meso-diaminopimelic acid (meso-DAP)-containing peptidoglycan of Gram-negative bacteria. Nod1 senses fragments of the stem peptides that terminate in meso-DAP, with or without MurNAc attached, and can also sense stem peptide fragments terminating in D-Ala in some hosts. Similarly, it is shown that *S. aureus* induces the selective autophagy and escape from the autophagosomes and proliferates in the cytoplasm. After invasion, *S. aureus* becomes ubiquitinated and recognized by receptor proteins such as

SQSTM1/p62 leading to phagophore recruitment [84]. Hypochlorous acid (HOCl) is produced by the host's phagocytic immune reaction by macrophages and neutrophils. It is very reactive and interacts with proteins and fatty acids of bacteria and suppresses DNA synthesis; but recently chlorination was reported to be involved in gaining chaperone activity of bacterial Hsp33, RidA, and DUK114 proteins. It has also been reported that chlorination of YabJ of *S. aureus* activates its ribonuclease activity [85] but little is known about chlorination and bacterial response.

Methods to study PTM

Studies demonstrated various techniques that are used to identify the post-translationally modified proteins and the specific site of modification. Mass-spectrometry-based proteomics is generally used to identify phosphorylation (phosphoproteomics) [86], glycosylation (glycoproteomics), oxidative damage to proteins (redox proteomics), ubiquitination (diglycine remnant proteomics), protein fragmentation (degradomics) and various other PTMs [87]. This facilitates the scientists to get a clear view of the molecular mechanism and regulation of the signaling pathways in multidrug-resistant strains and the wild-type, which might help to develop new antibiotics against the proteins involved in the signaling or metabolic pathways in resistant strains. Phosphoproteomics includes phosphopeptide enrichment (via cation exchange chromatography, titanium dioxide enrichment, hydrophilic interaction chromatography, immobilized metal affinity chromatography, antibody-based enrichment and many more), high accuracy mass spectrometry and related bioinformatics tools [86]. Li *et al.* suggested that, when photo-cross-linking and stable isotope labeling in cell culture (SILAC)-based quantitative mass spectrometry are used together, PTMs mediating protein–protein interactions like lysine methylation can be identified [88].

PTMs are targets for drug discovery

Bacterial tyrosine (BY) kinases can be considered as the superfamily of prokaryotic protein tyrosine kinases in bacteria [89,90]. These BY kinases do not have either sequence or structural homology with eukaryotic tyrosine kinases, and they phosphorylate tyrosine residues using an ATP/GTP-binding Walker motif. BY kinases take part in many different cellular processes, including DNA replication, sporulation, antibiotic resistance, heat-shock response, biofilm formation, and virulence. BY kinases lack the eukaryotic Hanks domain signature and have been identified in ESKAPE pathogens, for example, Ptk kinase of *Acinetobacter* [91] and PrkC in *Bacillus anthracis* involved in biofilm formation [92]. Histidine kinases also act in signal transduction pathways and are involved in bacterial two-component systems [93]. Inhibitors against histidine kinases have also been examined using structure-based approaches to develop novel antibacterials [94]. Recently, other kinases such as Asp kinases were reported in prokaryotes that also act as putative targets for drug resistance [50]. A recombinant vaccine has been generated against the N-glycosylation machinery of *Burkholderia pseudomallei* [95]. There are different approaches such as nanomedicine [6,7] or herbal [8–10,96] or *in silico* [11–14] methods that can be used to develop inhibitors against these bacterial kinases and other enzymes involved in PTM of ESKAPE pathogens, which could be useful to control ESKAPE pathogens. It has also been reported recently that cross-phosphorylation events (by bacterial serine/threonine and tyrosine kinases) could influence the capacity of recipient host kinases to phosphorylate substrates downstream in the signal transduction cascade [97]. Therefore, care should be taken in designing an inhibitor against the kinases that have cross-phosphorylation properties.

Concluding remarks and future perspectives

Recently, PTM was identified as having a crucial role not only in complex eukaryotes but also in bacterial systems. Studies have demonstrated the significance of various modifications in the maintenance of bacterial cellular metabolism, physiology, adaptability, and virulence. Bacterial pathogens are also reported to utilize PTMs, such as adenylation, glycosylation, acetylation or phosphorylation, to disrupt the stability and functionality of the host target proteins to facilitate their survival in the host during infection. Methionine cleavage and N-terminal acetylation are two common protein N-terminal modifications. Despite their importance in bacterial processes, they are less investigated. The characterization of N-terminal acetylation in bacteria is a challenge because no antibody exists, and it is a less frequent modification than in eukaryotes. Detailed proteomic studies of PTMs for the ESKAPE pathogens are essential to identify the host cell pathways that are targeted by the PTMs of bacteria and, thereafter, the role taken by PTMs in the infection strategy. These discoveries reveal the molecular basis of pathogenesis of the bacteria along with a clear understanding of the eukaryotic PTMs and the complex cellular pathways associated with the infection. Although mammalian-based studies of infection are limited owing to ethical issues, it is crucial to have an improved mechanistic knowledge in a proper *in vivo* setup. PTMs play an essential part for these nosocomial pathogens either in their own cellular system or in that of the host for their virulence and pathogenicity. The identification and study of different PTMs, and proteins manipulated by these PTMs, provide better insight of the host–pathogen relationship. Targeting the bacterial PTMs that are involved in pathogenesis might help the researchers to prevent the spread of ESKAPE pathogens and could be used as one of the strategies for controlling the spread of virulence of these bacteria.

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Conflicts of interest

The author has no conflicts of interest to declare.

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