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Proteomic analysis of bacterial communities associated with atopic dermatitis

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ABSTRACT

Atopic dermatitis (AD) is a relapsing, chronic, and inflammatory skin disorder. Its causes remain unclear. Here, we reported the first proteome study of the bacterial community in AD patients. Bacterial community in 7 patients and 1 healthy control using bottom-up proteomics were examined starting with in-solution digestion followed by purification steps with subsequent analysis using LC-MS/MS and ended with data processing and bioinformatic analysis. Overall, great bacterial changes between patient samples and healthy one were noticed with the presence of *Staphylococcus aureus, Aeromonas hydrophila,* and Shewanella species, and others that were present uniquely in patient samples suggesting their role in AD. Additionally, detection of some important proteins that trigger bacterial pathogenesis and the immune system such as enolase, glyceraldehyde-3-phosphate, Chaperone proteins DnaK and HtpG beside protein pathways needed for bacterial growth and pathogenesis like chaperones and folding catalysts; and Energy metabolism. These new findings of the microbiome and detected proteins could start a new era of proteomics to study the bacterial community as a whole and detect the way it interacts with each other and with the host.

Significance: This paper would represent a reference work for investigations on microbiota that present on AD, from both a microbiological and a functional proteomic point of view. We focused on analysisng bacteria community and proteins produced and its role in the disease, highlighting some functional characteristics of certain proteins and discussing its potential role in AD.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease that affects children worldwide. 10% to 20% of children have AD in the United States and approximately 25% of children around the world have AD [1,2]. It is characterized by pruritus and dry skin in addition to crusted, red, and relapsing skin lesions [3]. It is usually connected to asthma, food allergy, and allergic rhinitis. The AD patient suffers from sleep disorders, skin infections, and psychosocial morbidities including anxiety and depression [4,5]. The cause of AD is incompletely known. Some researchers focused on causing factors that related to a human while others focused on the skin microbiome. The first group related AD to the immune system and skin composition especially filaggrin (skin barrier protein). A decreased expression of filaggrin was obviously reported in AD patient's skin [6]. That was confirmed after the detection of the mutation in the filaggrin gene (FLG) [7]. However, this does not mean that all AD patients carry FLG mutation. Yet, FLG mutation has been related to childhood-onset of AD, especially 2 years old children or younger [8]. On the other hand, A great change in the immune system was detected in AD patients starting from the innate to the adaptive immune system. A remarkable decrease in antimicrobial peptides; sphingosine, dermcidin, cathelicidin, and β-defensins were noticed in AD skin patients [9–11]. In addition to the first line immunity, a remarkable increase in thymic stromal lymphopoietin (TSLP) produced by keratinocytes which lead to increasing CCL17 and CCL20 chemokines secreted by macrophage and dendritic cells. This chemokine increases T-helper 2 cell (Th2) infiltration and its expressed cytokines; IL-4 and IL-13 [12,13] which decrease AMP production, filaggrin expression, and increase *S aureus* attachment to AD skin [14–16]. All these changes in the innate and adaptive immune system lead to increasing the susceptibility of AD to infection and increase the severity of the disease.

The second group related AD to the microbiome and pathogenic bacteria especially *Staphylococcus aureus*. A great change of microbial

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community in the AD skin patient has been noticed. The proportion of some bacterial species were found to decrease during the flare time; Corynebacterium, Streptococcus, and Propionibacterium species. *S. epidermidis* prportion during flare time was significantly higher than during postflares. In contrast, staphylococcal species proportions were found to increase especially *S aureus* [17]. *S aureus* cell wall components were found to trigger the production of epidermal thymic stromal lymphopoietin (TSLP) by keratinocytes and its following sequences [12]. Alpha toxin produced by *S aureus* was found to activate T cell for the production of interferon (IFN)-ϒ and induce keratinocyte cytotoxicity that may lead to chronic AD [18].

For more information about the bacterial role in AD patients, many genomic researches were focused on bacterial distribution in AD lesions [17]. Although DNA and RNA sequences have already been known, it does not reflect the temporal and spatial proteome of bacterial communities associated with AD [19].

In the past, two-dimensional polyacrylamide gel electrophoresis (2D PAGE) trailed by MS analysis has been used as the chief way for proteomic analysis. It was used to study bacterial pathogens associated with many skin diseases [20,21]. At the beginning, it seemed that protein identification by MS help 2D PAGE analysis, but it showed that thousands of spots noticed in the gel maps are in fact variants of the most abundant proteins [22]. Besides, protein quantitation is not accurate because of spot overlap [23]. In addition, it has some disadvantages mainly toward the identification of very low and high molecular weight proteins and proteins with low abundance besides being time-consuming technique. For these reasons, new approaches have been developed like gel free MS-based approach known as gel-free shotgun proteomics [24]. Proteomics-based on mass spectrometry (MS) has the ability to study proteins and how they interact with each other. Which in turn helps us to understand mechanisms of antibiotic resistance, dysregulations that occur during infection, and also to find new targets for drug discovery in the future [25,26]. Proteomics also required for identifying proteins and their pathway during infection, That could help us to know the interaction between bacterial community itself and between human and microbiome [27].

The use of newer mass spectrometers generations accompanied by high-performance liquid chromatography (HPLC) enables us for more protein separation followed by better detection and identification [27]. In addition, in-solution digestion is favored over in-gel digestion because it is more power over the outcome with steps less than in-gel digestion steps, this advantage helps us for more peptide yield with a lower loss between steps. Therefore, it is preferred when the bacterial load is small. More control over the conditions e.g. pH, digestion buffer, proteolytic enzymes, protein concentration is easier in case of in-solution digestion [28,29].

Since the proteomic analysis is useful for providing a lot of information about proteins and protein-protein interactions and since most studies of AD were focused on the genome level. In this study, we aimed to perform a proteomic analysis of the bacterial community associated with AD in Egyptian patients for the first time aiming for a wide perspective to identifythe mechanism of critical proteins produced by bacterial community to affect the skin in the lesion site during AD.

2. Materials and methods

2.1. Samples collection

Samples were collected from the skin of patients with a confirmed diagnosis of severe to moderate on the basis of the Hanifin and Rajka criteria [30]. Clinical severity of AD was assessed using the Scoring AD (SCORAD) index [31]. According to the SCORAD, patients were categorized into three groups; mild, moderate, and severe. We targeted AD patients with ages ranging from 2 to 40 years old. Sampling was done using skin scraping technique in cryotubes [32]. Any patient receiving chemotherapy or radiation treatment for malignancies within the previous 6 months and any patient who was unable to remain off systemic antibiotics or systemic steroids for at least 7 days prior to sampling were excluded. Healthy control subject was nonatopic, within the age range and lack of major skin diseases, including psoriasis, rosacea, and previous AD. Each sample was stored at −80 °C until use.

2.2. Protein extraction and digestion

Protein extraction was performed by adding 150 μl of 2 M urea buffer (pH 8.5) to each sample and then incubated overnight at room temperature. Eighty μl from each sample were mixed with 60 μl of 0.2% formic acid. One microliter of 1 M tris carboxyethyl phosphine (TCEP) was added, samples were vortexed and spun down for volume reduction. The samples were incubated for 30 min. at room temperature [33]. Proteins were alkylated by adding 2.4 μl of 1 M iodoacetamide (IAA) and incubated at room temperature for 1 h in dark. Six μl trypsin containing 1 μg procaine enzyme was added to the tube for trypsinization. The tube was incubated overnight at 37 °C with shaking at 600 rpm. Formic acid (6 μls of 100%) was added to acidify the sample to pH 2–3. The tube spun down for 30 min. at maximum speed at room temperature.

2.3. Protein purification

Stage tip (pierce ™ C 18 spin tips) was used with centrifugation between each step at 3000 rpm. Tips were activated by adding 15 μl methanol and initialized with 15 μl from solution B (0.2% formic acid (FA) +80% acetonitrile (ACN)) were added, and for re-equilibration, 15 μl from solution A (0.2% FA) were added twice. Samples were trapped and washed with 15 μl of solution (A) twice [34]. Elution was performed in a collection tube, 3 times each 20 μl of solution (B) were added. Speed vacuum of the samples was carried out and the samples were re-constituted in 70 μl from solution (A).

2.4. LC-MS/MS analysis

Bottom-Up proteomics was performed using LC-QTOF Mass spectrometry (Sciex TripleTOF ™ 5600+) preceded by NanoLC system consisting of Eksigent nanoLC 400 autosampler attached with Ekspert nanoLC425 pump for peptide separation and analysis. Information-dependent acquisition (IDA) parameters were set to detect the most intense 40 ion of the High resolution TOF MS survey scan followed by product ion scan. In addition, the TOF mass range and MS2 range were 400–1250 m/z and 170–1500 m/z respectively. About 0.5-microgram peptide were injected for 55 min using trap and elute technology. Sample clean-up using trapping cartridge CHROMXP C18CL 5 μm (10 × 0.5 mm) pumped at a flow rate of 10 μl/min for 3 min using mobile phase A (DI-Water containing 0.1% FA). Elute using 3 μm ChromXP C18CL, 120A, 150 × 0.3 mm column with 5 μl/min flow rate. Two mobile phases were phase A (DI-Water containing 0.1% FA) and solution B (ACN containing 0.1%FA). Elution starts with 97% and 3% and ends with 20% and 80% of solution A and B, respectively [35].

2.5. Data processing

Analyst TF 1.7.1 was used for data acquisition (Sciex software). Protein pilot (version 5.0.1) analyzed raw MS files from the TripleTOF ™ 5600+, paragon Algorithm (version 5.0.1.0, 4874). The database used is the UniProt Bacteria database (containing 83,980 proteins).

2.6. Bioinformatic analysis

Identified proteins were searched for biological functions according to gene ontology annotation (GO) to explore biological functions, its role in bacterial pathogenesis, and finally to determine the effect of protein on the immune system using the UniProtKB database (www.

uniprot.org). Pathological and immunological role of the proteins were detected using The Entrez database (www.ncbi.nih.gov). Finally, the KEGG database (www.genome.jp/kegg) was used to determine possible pathways of proteins.

3. Results

A proteomic analysis was carried out to survey the bacterial community associated with AD skin. Seven skin scraping samples were involved in our study. MS/MS technique identified 778, 124, 183, 57, 622, 63, and 271 proteins in the 7 samples. These identified proteins were subjected to manual refinement by removing the duplication of proteins with the same function even if they are from a different bacterial source. This refinement revealed 92, 58, 90, 7, 18, 13, and 15 proteins; respectively, 219 proteins collectively as illustrated in Supplementary Table 1. Gene ontology of proteins revealed that these proteins were involved in different bacterial functions e.g., replication, growth, and virulence. Some of these proteins play an important role in bacterial pathogenesis and adhesion such as cytadherence a high molecular weight protein, vacuolating cytotoxin autotransporter, and alpha-glycerophosphate oxidase. Other proteins were found to affect the immune system such as hemolysin A, malate dehydrogenase, and enolase.

Moreover, some proteins were found to be identified with a massive number in many bacteria e.g.; ATP synthase was represented by 526 peptide sequences from different bacteria as appeared in sample 1. Chaperone protein DnaK was represented by 132 peptide sequences from different bacteria as in sample 5 as shown in Table 1.

On the other side, we have found a numerous number of shared bacteria participating in the AD. Supplementary Table 2 showed the different types of bacteria that were found to be shared in at least 3 patient samples. Fortunately, these bacteria were not represented in the control healthy sample.

Collectively, we found 84 bacterial species shared in 3 samples, 34 in 4 samples, 12 in 5 samples, and 8 in 6 samples. The most significant types that appeared to be common in approximately all AD patient samples were *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Alcanivorax borkumensis, Bifidobacterium animalis, Caulobacter crescentus, Chromohalobacter salexigens, Clostridium acetobutylicum, Pseudoalteromonas haloplanktis, Rickettsia akari, Serratia proteamaculan, Shewanella halifaxensis, Shewanella pealeana,* and *Shewanella woodyi*.

Samples 1, 5, and 7 have the greatest percentage of shared bacteria, while samples 4 and 6 have the least one (Fig. 1).

The phylum distribution over samples indicates that the persistence of proteobacteria in both healthy and patient samples (Fig. 2 and Table 2). While actinobacteria, cyanobacteria, and firmicutes increase in patient over healthy samples. In contrast, Fusobacterial phylum was found in healthy more than patient samples.

Bacterial genera were observed in our samples mainly Campylobacter, Escherichia, Haemophilus, Pseudomonas, Rhizobium, Rickettsia, Salmonella, Shewanella, Shigella, Staphylococcus, Streptococcus, Vibrio, and Yersinia. Staphylococcus, Streptococcus, Campylobacter, and Shewanella were mainly in patient with a higher percentage over healthy sample. On the contrary, Escherichia and Haemophilus are a little bit more in healthy sample. Yet, some genera were approximately the same in both patient and healthy samples like Yersinia and Shigella (Fig. 3).

Concerning species level of Staphylococcus and Streptococcus, the obvious presence of proteins related to *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,* and *Streptococcus pyogenes* was only found in the patient samples (Table 3 and Fig. 4).

KEGG database was used to detect protein functions and its possible pathways. Briefly, in AD patients there were 43 and 66 protein pathways were enriched at level 2 and level 3, respectively (Fig. 5 A & B, respectively). Remarkable availability of certain pathways like translation, amino acid metabolism, carbohydrate metabolism, energy metabolism, and nucleotide metabolism were noticed. In addition to other protein pathways associated with pathogenesis such as chaperones and folding catalyst; antibiotic resistance; drug resistance; and peptidases. Amino acid metabolism pathway includes phenylalanine metabolism; Arginine and proline metabolism; histidine metabolism; Glycine, serine, and threonine metabolism; Cysteine and methionine metabolism; and D-arginine and D-ornithine metabolism pathways. (Supplementary Tables 3 and 4).

4. Discussion

Former studies concerning atopic dermatitis (AD) had been focused on two different aspects: human and bacteria. Proteomic analysis of AD skin showed alteration in proteins involved in skin barrier, natural moisturizing factors, in addition to increase in epidermal fatty acid-binding protein expression in methicillin resistant *Staphylococcus aureus* skin lesions [36]. Most of the studies concerning bacteria were concentrated on the most prominent bacteria; *Staphylococcus aureus* and its role in the AD. Others pointed the whole bacterial community but only from genetic bases which could not explain protein expression. Here we targeted the bacterial community associated with AD on proteomic base comparing them with that of a healthy individual.

We identified 219 proteins in the AD patient samples, many proteins were found to be responsible for normal bacterial functions such as growth, replication, and pathogenesis. Others were found to affect the host immune system. Some proteins were found to share multiple functions. These proteins help in the bacterial invasion and adhesion and thus increase the severity of AD flare. Interestingly, one of these proteins was enolase which is identified in our list of proteins found exclusively in AD samples. It is a glycolytic protein present on the surface of many pathogenic bacteria like *Aeromonas hydrophila* and *Staphylococcus aureus*. Glycolytic protein facilitates plasminogen attachment to a bacterial surface, which helps in bacterial adhesion and tissue invasion turning the bacteria to a proteolytic one [37,38]. It was confirmed previously that enolase plays a critical role in the immune response against AD. *Streptococcus pneumonia* enolase induces the formation of neutrophil extracellular traps (NETs) [39]. Enolase

Table 1
The list of proteins that repeated with massive no. in samples.

Protein name	Repeatition number						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
ATP synthase	526	4	9		306		
Chaperone protein DnaK	2		4	3	132		134
Chaperone protein HtpG	13				13		13
Glyceraldehyde-3-phosphate dehydrogenase	17	9					16
Leucine–tRNA ligase	40			42	40	40	41
Ribonuclease Y	8	8					
Malate dehydrogenase					34		34
Nucleoside diphosphate kinase					21		21

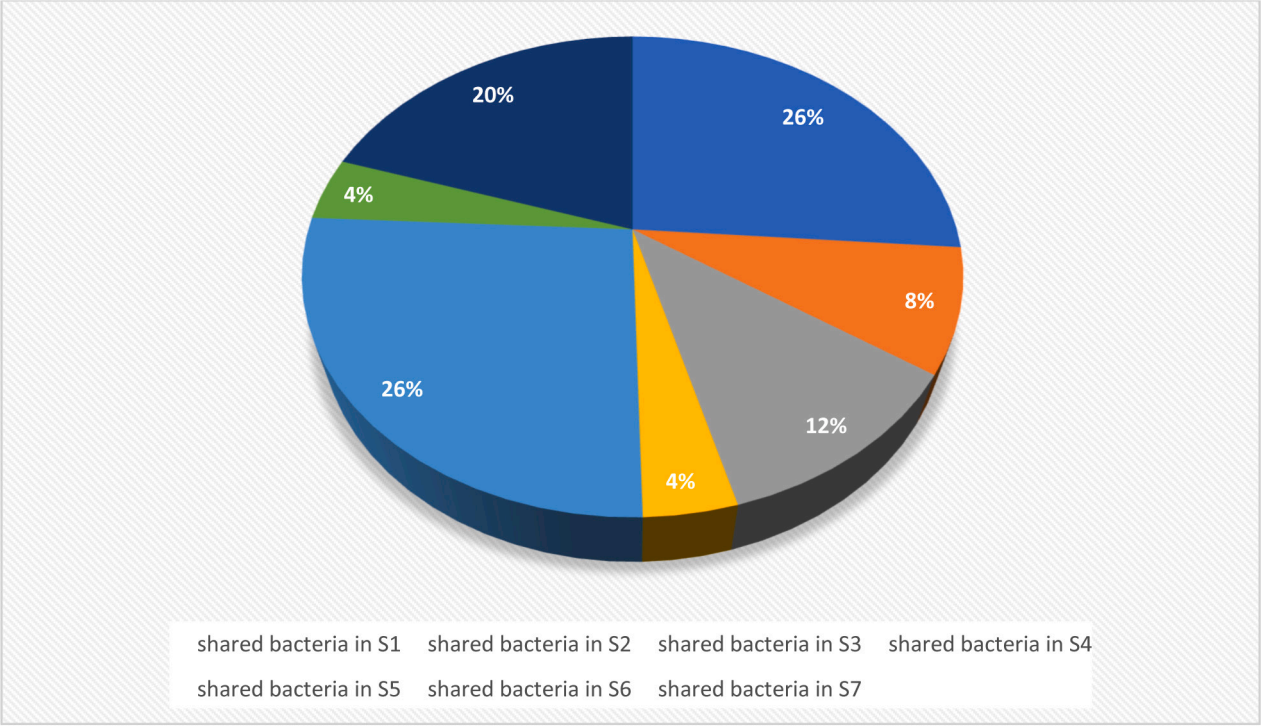

Fig. 1. Contribution of each sample to shared bacteria.

recombinant with *Streptococcus sobrinus* suppresses the immune response against T-cell dependent antigens. It enhances the production of anti-inflammatory interleukin-10 [40]. This reveals how enolase suppresses the immune system. Herein we identified enolase with a high score in *Hydrogenovibrio crunogenus*.

Coincidently, we identified Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) massively in AD samples. It is another glycolytic enzyme, reacts in the same manner as enolase via binding to plasminogen [41]. GAPDH also interacts with the immune system in different ways. GAPDH of *Streptococcus agalactiae* was found to increase interleukin-10 which suppresses antigen-presenting cells [42]. It also interrupts the complement system via trapping of C5a, a chemical mediator of neutrophils [43]. GAPDH was also found to mediate cell surface adhesion, the first step of infection [44]. At this point we identified GAPDH with a high score and a massive amount in *S. aureus*. Owing to these results of identifying both enolase and GAPDH in AD

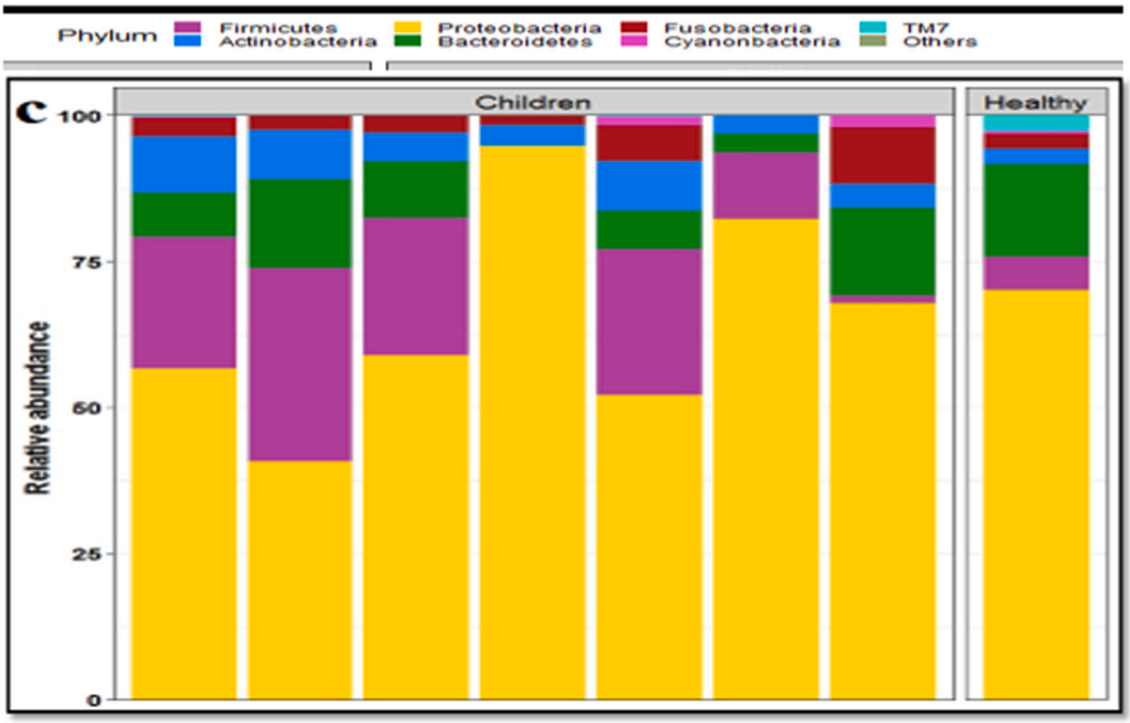

Fig. 2. Phylum distribution over samples included in our study.

Table 2
Phylum distribution over samples used in our study.

Phylum	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Healthy
Actinobacteria	57	10	8	2	40	2	10	5
Bacteroidetes	1	0	0	0	7	0	5	1
Cyanobacteria	20	3	5	1	30	0	23	5
Firmicutes	136	39	39	0	120	7	3	11
Fusobacteria	1	0	0	0	1	0	0	5
Proteobacteria	339	48	97	53	249	51	162	133
Others	46	18	16	0	32	2	36	30

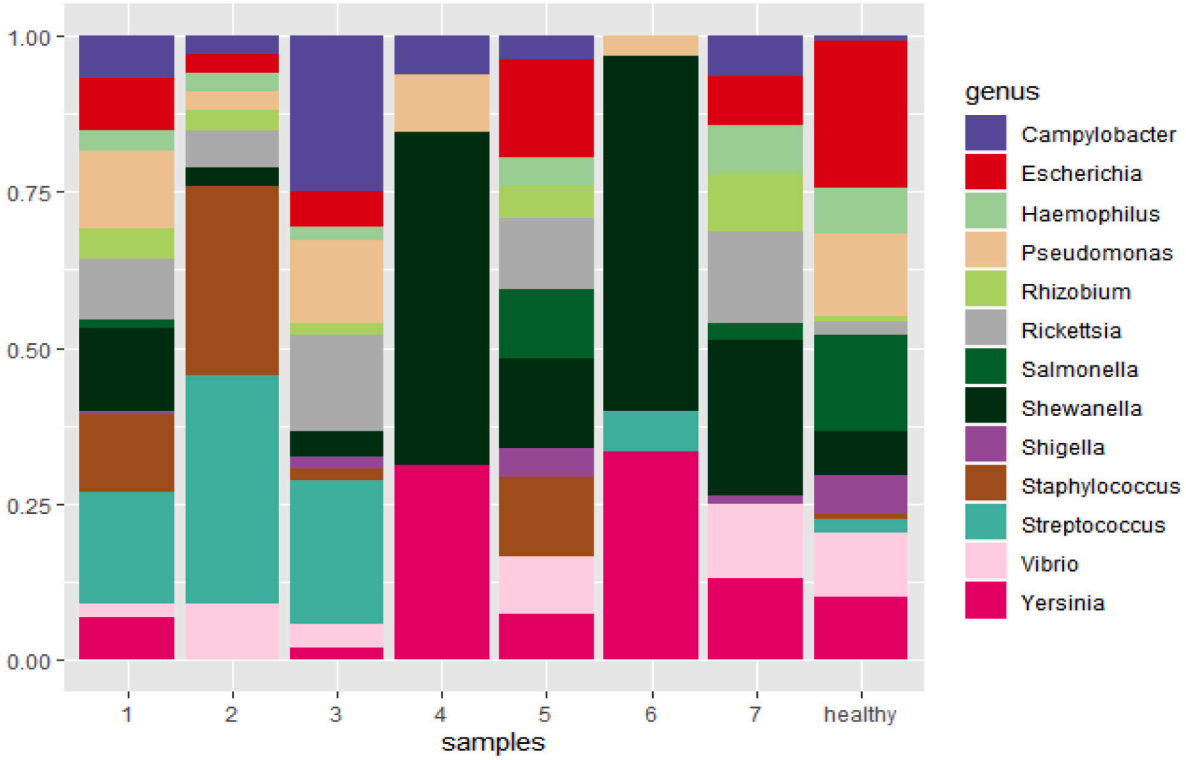

Fig. 3. Genus distribution over samples included in our study.

Table 3
Species level distribution of staphylococcus and streptococcus.

Species	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Healthy
Staphylococcus aureus	14	7	0	0	13	0	0	0
Staphylococcus epidermidis	2	2	0	0	2	0	0	0
Staphylococcus haemolyticus	1	1	1	0	1	0	0	0
Staphylococcus saprophyticus	1	0	0	0	1	0	0	1
Streptococcus agalactiae	3	0	0	0	0	0	0	0
Streptococcus equi	3	0	0	0	0	0	0	0
Streptococcus mutans	1	0	1	0	0	0	0	1
Streptococcus pyogenes	12	12	11	0	0	0	0	0
Streptococcus suis	2	0	0	0	0	1	0	0
Streptococcus thermophilus	3	0	0	0	0	0	0	0
Streptococcus uberis	1	0	0	0	0	0	0	0

samples, we could correlate in a more robust fashion their roles in the pathogenesis of AD.

Moreover, we could detect many proteins that help bacteria to survive from environmental conditions and immune system attacks and this aid in increasing the pathogenicity of the bacteria. Some of these proteins were 10 kDa chaperonin (cpn10), chaperone protein HtpG, peroxiredoxin 2, putative peroxiredoxin and multidrug-efflux transporter.

Chaperone proteins (heat shock proteins), DnaK and HtpG, are the main classes of heat shock protein that were identified in AD samples in *Corynebacterium efficiens* and *Rickettsia typhi,* respectively (Supplementary Table 1). DnaK is noticed to be expressed on the bacterial cell surface and can be detached to the extracellular environment, so act as a virulence signal and interact with an immune attack during AD different stages [45]. In this study, DnaK was represented by 132 peptide sequence from different bacteria as appeared in sample 5 (Table 1). Chaperonins like 60 kDa chaperonin (Cpn 60) and 10 kDa chaperonin (Cpn 10) were also identified in the AD patients' samples

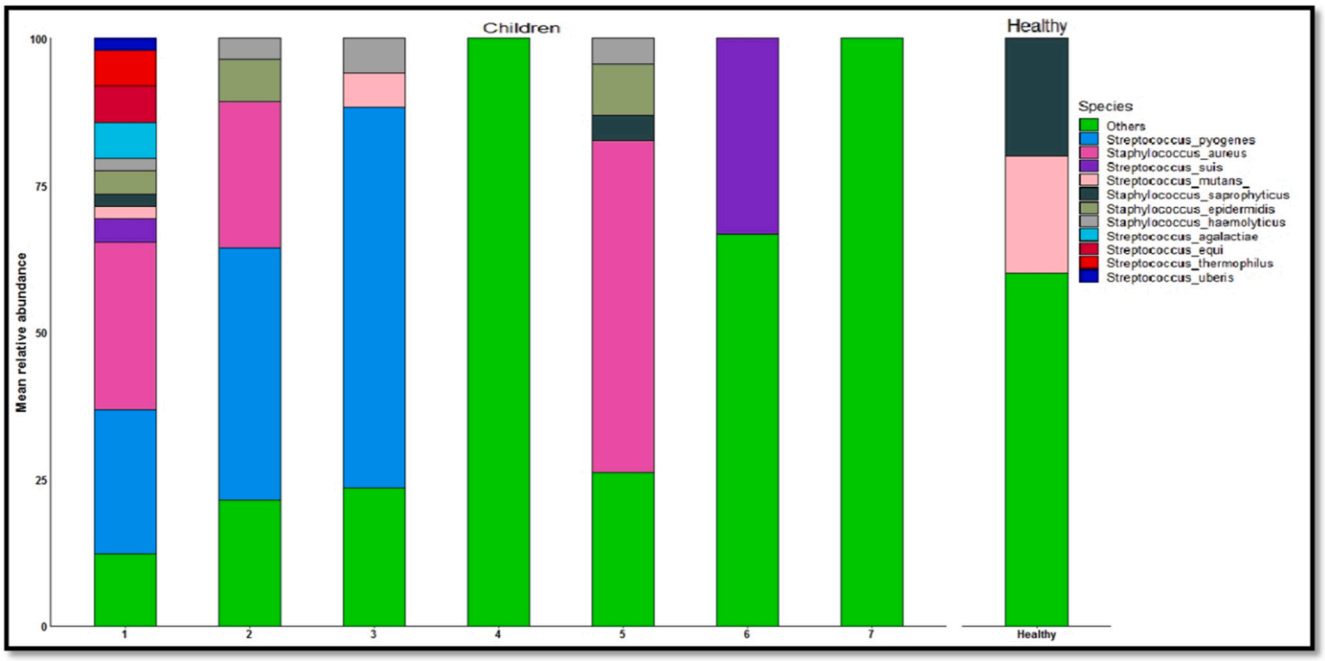

Fig. 4. Species level distribution of Staphylococcus and Streptococcus.

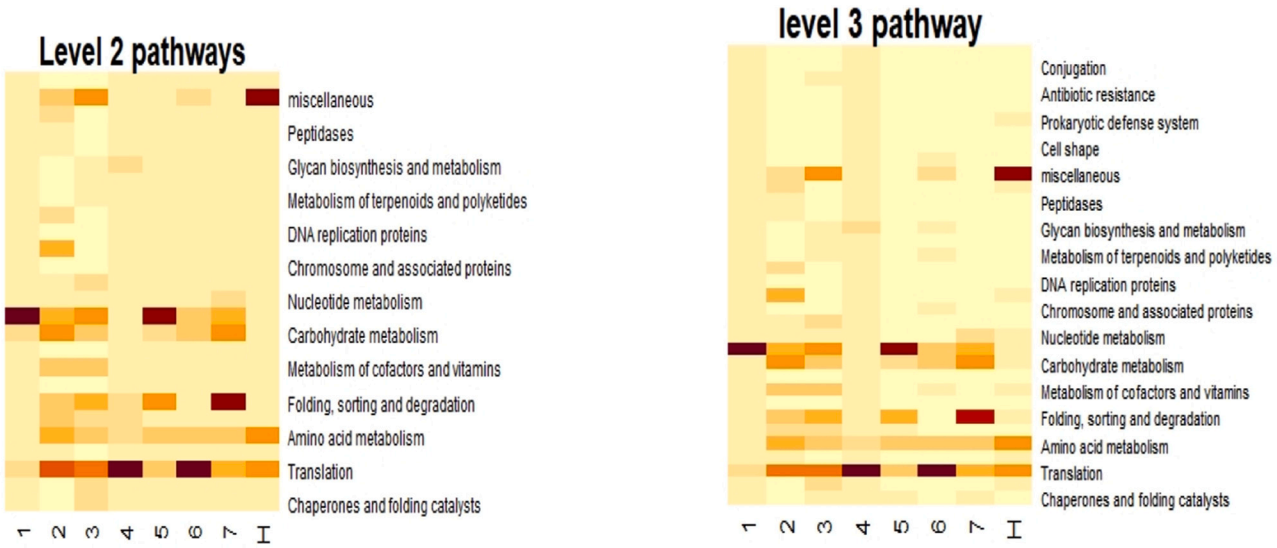

Fig. 5. heatmap show level 2 & level 3 pathways distribution.

with a high score in *Xanthomonas campestris* and *Rickettsia typhi*, respectively. Cpn 60 is an immunogenic protein that stimulates the acquired and innate immune system and this can be illustrated by the presence of activated T lymphocytes that respond to mycobacterial Cpn 60 in *Mycobacterium tuberculosis*-infected mice [46]. While Peroxiredoxin 2, Putative peroxiredoxin, and Catalase-peroxidase help bacteria to reduce H_2O_2, consequently, it helps bacterial survival from macrophage attack and oxidative burst [47–49]. Multidrug-efflux transporter is another protein that identified in *Bacillus subtilis* in our samples. It confers bacterial pathogenesis with a different mechanism and increases bacterial resistance via the elimination of antibiotics from bacteria [50].

On the other hand, some proteins were found to enhance attachment and colonization and thus increase bacterial pathogenesis. Precisely, Alpha-glycerophosphate oxidase (GlpO) was identified in our samples (Supplementary Table 1). It was proven that GlpO facilitates *Streptococcus pneumoniae* attachment to the nasopharynx [51]. It also helps bacteria to produce H_2O_2, which increases bacterial pathogenesis. Another protein, Cytadherence high molecular weight protein 2, also required for attachment of *Mycoplasma pneumoniae* to the cell surface [52,53]. It was clearly found in the AD patient samples.

Other proteins that help bacterial pathogenesis and were luckily identified in our AD patient protein list were lipid-A-disaccharide synthase, methionyl-tRNA formyltransferase, prolipoprotein diacylglyceryl transferase, vacuolating cytotoxin autotransporter, nucleoside diphosphate kinase, hemolysin A, and malate dehydrogenase. Methionyl-tRNA formyltransferase (FMT) in mutated bacteria showed an apparently reduced growth rate with decreased production of exotoxins and other virulence factors that led to a decrease in its pathogenicity. Ninty percentage of animals infected with FMT mutant *S. aureus* were found to survive while that infected with wild-type were died [54]. This shed the light on the importance of this proteins in the AD pathogenesis.

Nucleoside diphosphate kinase (Ndk) is a housekeeping enzyme, which plays a role in bacterial virulence. Intracellular Bacterial Ndk

secreted suppress host defense mechanisms like apoptosis and phagocytosis. While extracellular Ndk exaggerates the inflammatory response [55]. Malate dehydrogenase (MDH) is an important protein concerning bacterial virulence. In vivo studies on MDH of *Brucella abortus* showed that it was found to be an immunogenic protein. It can bind to plasminogen and fibronectin and this may explain its role concerning invasion and adhesion [56]. The presence of these proteins and their roles in bacterial adhesion, pathogenesis and its interaction with the immune system, gives us an indication about their contribution in increasing the severity of AD flare in addition to increasing bacterial colonization of pathogenic bacteria to the skin flare.

From another point of view, we have noticed an obvious repetition of some proteins as shown in Table 1. ATP synthase was repeated 526, 4, 9 and 306 in samples 1, 2, 3 and 5 respectively. In samples 1 and 5, we could see a massive number of ATP synthase. This was also clear concerning Chaperone protein DnaK in samples 5 and 7 with 132 and 134 repetitions respectively. This massive production of specifically these proteins indicates its importance. ATP synthase is an important protein and required for energy production and bacterial growth. The blockage of ATP synthase function dramatically diminishes growth via depletion of cellular ATP levels and thus bacterial killing [57,58].

Other proteins were approximately repeated with the same numbers in different samples by the same bacteria as Chaperone protein HtpG, Leucine–tRNA ligase, Ribonuclease Y, Malate dehydrogenase and Nucleoside diphosphate kinase. It was clear that all these proteins were repeated approximately with the same number. Every repeated protein has the same amino acid sequence, the same entry name and also expressed by the same bacteria in parallel in different samples as illustrated in Supplementary Table 5, for example, Chaperone protein HtpG protein which repeated 13 times in samples 1, 5 and 7. All these repeated proteins with a massive number might have a role in bacterial pathogenesis and AD lesion. Also, the repetition of the same protein in different samples from the same bacteria gives us an indication of the importance of these bacteria and proteins in AD. Further studies are needed to investigate the exact role of these proteins in AD.

On the other hand, a lot of bacteria were found to be shared in more than one sample as illustrated in Supplementary Table 2. Some of these bacteria were already confirmed to have a role in AD such as *Staphylococcus aureus* and *Staphylococcus epidermidis* [17]. Other bacteria have no confirmed role in AD, but present in at least three patient samples and fortunately, not represented by a single protein in the healthy sample. This may give us proof about the role of these bacteria in AD. In as much, we found two Aeromonas species that present in six patients. The previous study found that Aeromonas implicated to cause skin and wound infection [59] but unfortunately its role in AD has not been declared yet and still needs mor investigation. This may support our finding that Aeromonas may have a role in AD disease. Other bacteria that were also found in AD samples and could cause skin infection were *Bacillus anthracis*, *Bacillus cereus,* and *Streptococcus pyogenes*. Several studies disclosed that *Bacillus anthracis* is the causative agent of cutaneous anthrax [60], *Bacillus cereus* can cause cutaneous infection [61], and *Streptococcus pyogenes* is a common pathogen that causes impetigo, scarlet fever, cellulitis, pharyngitis, and toxic shock syndrome [62]. Additional pathogenic bacteria were also found in patient samples that known to cause many diseases. Four Bartonella species; the causative agent of cat scratch disease [63], four Campylobacter species; the main reason for acute diarrhea and gastroenteritis [64], three clostridium species; which is not an invasive bacteria but it produces powerful toxins like botulinum toxin that causes botulism [65], *Mycoplasma genitalium*; that cause urethritis in men and in women [66], and numerous Rickettsia species. Here we identified the most important bacteria in AD; *S. aureus* which implicated in the skin infection starting from impetigo, boils, erythema, cellulitis, and abscess to the life-threatening necrotizing fasciitis [67]. Concerning AD, *S. aureus* role is so obvious as the major microorganism present in AD flare [17]. Many reports proved the high relevance between AD and the presence of *S. aureus* [68,69].

Intriguingly, various protein pathways that needed for continuous bacterial growth and pathogenesis were shown in Supplementary Tables 3 and 4. Most important for bacterial growth were amino acid metabolism, carbohydrate metabolism, energy metabolism, and pathways that contribute to bacterial replication like DNA repair and recombination proteins, chromosome and associated proteins. Other pathways shared in pathogenesis were chaperones and folding catalysts, Energy metabolism, Biosynthesis of other secondary metabolites, Drug resistance, Peptidases, and Signal transduction. This is in agreement with a plethora of former studies that correlate the bacterial community pathways with the growth and pathogenesis [70–72].

On the other hand, protein pathways that alter the pH were also detected (Fig. 5). Change of skin pH is one of the main factors that interfere with AD mechanism [73]. Many protein pathways that increase the pH like arginine and proline metabolism; and phenylalanine metabolism pathways via ammonia production were previously established [74]. Consequently, pH elevation accelerates cascades of variations in the epidermal barrier which helps in AD progression.

5. Conclusion

In conclusion, proteomic analysis showed us important proteins produced by the bacterial community in AD at the time of the disease. Some proteins were found to have a role in bacterial pathogenesis and others interact with the immune system. Further investigations are needed to determine the exact role of these proteins in AD especially, enolase and GAPDH. On the other hand, the presence of shared bacteria uniquely in patient samples may conclude the role of these bacteria in AD.

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Ethical approval

This study was approved by Suez Canal University, Egypt ethical board. Permission and informed consent to collect skin samples were obtained from patients included in the study.

Author contribution

A.K. performed experiments, analysed the data, and wrote parts of the manuscript. A.H. validated data, conceived, analysed the data, review the manuscript and supervised the project. M.A. validate data, review the manuscript, supervised part of the project. S.E. analysed part of the data, review the manuscript, supervised part of the project.

Declaration of Competing Interest

The authors declare that they have no competing interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jprot.2020.103944.

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