Research Journal of Medical Sciences 11 (2): 103-113, 2017
ISSN: 1815-9346

Plesiomonas shigelloides Seventy Years of Systematics and Taxonomy in Perspective of the Present-Day Diagnostic Demands

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Abstract: The pathogen *Plesiomonas shigelloides* was discovered first in 1947 and christened C27 paracolon. Ever since then, it has suffered several taxonomic transitions. Although, throughout the world, the bacterium is highly recognized as public health threat both in human and veterinary medicines particularly in immunocompromised health, its studies and diagnostic development could be termed lingering among similar pathogens. The limitations of the existing taxonomic and diagnostic procedures were fully acknowledged. Emphasis was placed on the need for development of novel and rapid culture-independent diagnostic probes/protocols for its strain-specific (including pathogenic and nonpathogenic strains) characterization to enhance infection managements and possibility of having a unique identification scheme that is all-encompassing without bias towards some strains. Techniques with eminent congruence, specificity and sensitivity in characterizing its diverse strains were also advocated.

Key words: *Plesiomonas shigelloides*, public health, systematics, infection, diagnosis

INTRODUCTION

The *Plesiomonas* belongs to the family *Enterobacteriaceae* and has the facultative fermentative anaerobe *Plesiomonas shigelloides*, a gram-negative polar flagellate bacillus as its only member (Kaszowska *et al*., 2016). The genus has suffered several translations from one taxonomic group to another over the years. Briefly, the earliest description of the organism came from Ferguson and Henderson (1947) and was named C27 paracolon. Later, a four biotyping scheme emerged based on the research by Schmid from fermentative characteristics of lactose, dulcitol and salicin, followed by placement into genus *Pseudomonas* by Bader (1954). Its characteristic cytochrome oxidase and polar flagellation saw it into genus *Aeromonas*, in the *Vibrionaceae* family (Ewing *et al*., 1961). Finally, the researches by Habs and Schubert (1962), Martinez-Murcia *et al.* (1992), Ruimy *et al.* (1994), Huys and Swings (1999) and Garrity *et al.* (2001) chronologically saw it to the present genus *Plesiomonas* in the *Enterobacteriaceae* as the solely oxidase-positive member. Although, with the advent of different high throughput molecular techniques such as rRNA (5S, 16S and 23S) sequence-based techniques that placed *P. shigelloides* closely to Proteaceae via phylogenetic alignment, the taxonomic name remainstill today (Martinez-Murcia *et al*., 1992). A summary of *P. shigelloides* taxonomic transitions is presented in Table 1.

Morpho-phenotypically, *P. shigelloides* is a long straight motile rod, either polarly or peritrichously flagellated (2-8 flagella) with fermentative ability under facultative anaerobic conditions (Inoue *et al*., 1991). A typical plesiomonad cell is 0.70-1.0 μm by 2.10-3.00 μm sized (Gonzalez, 2003). Transmission electron microscopy of plesiomonad cells have shown the possession of inclusion bodies that composed polyphosphates granules (Ogawa and Amano, 1987; Pastian and Bromel, 1984). Fermentative characteristics of *P. shigelloides* ranged from acid but non-gas production from glucose, inositol, ornithine to lysine decarboxylation. It is an active producer of β-galactosidase, phenylalanine deaminase, elastase (hydrolyze elastin), arginine dihydrolase, ornithine decarboxylase, trehalase, lysine decarboxylase, cytochrome oxidase, maltase, chitinase and DNase (hydrolyze chitin) but unable to produced starch

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Table 1: Summary of *P.Shigelloides* taxonomic transition over the year

Taxonomic name/transition	Reasons or characteristics for placement	References
C27	Motile enteric, anaerogenic, paracolon, amphitrichous organism, *Enterobacteriaceae*, somatic antigen similar to Shigella sonnei Phase 1	Ferguson and Henderson (1947) Schmid
Pseudomonas shigelloides	Isolated from faeces, gram-negative rods have somatic antigen like Shigella sonnei, Polar or lophotrichous flagellation, anaerogenic glucose fermenting, non- lactose fermenting, nitrate reducer (nitrate to nitrite)	Bader (1954)
Pseudomonas michigani	Lophotrichous flagellated gram-negative bacilli; and based on geographic pedigree where Ferguson and Henderson isolated this bacterium	Sakazaki *et al.* (1959)
Aeromonas shigelloides	Sugar fermentative ability compared with oxidative activity, cytochrome oxidase positivity	Hugh and Leifson (1953), Gaby and Hadley (1957)
Pseudomonas, aeromonas or vibrio	Based on gram-negative bacteria polar flagella differentiation	Ewing and Johnson (1960), Ewing *et al.* (1961), Shewan *et al.* (1954), Shewan *et al.* (1960), Shewan (1963)
Pseudomonas shigelloides, *Pseudomonadaceae* in the tribe pseudomonadeae	Based on flagellation and morpho-phenotypical backdrops, only glucose-fermenting anaerobe in the family	Habs and Schubert (1962)
Plesiomonas (proposed)	Based on comparative studies with aeromonas and vibrio	Sabald and Veron (1963)
Fergusonia (proposed)	Comparative Guanine and Cytosine (GC) deoxyribonucleic acid content of Aeromonas, Vibrio, Pseudomonas, Moraxella; Pseudomonas (64%), Aeromonas (60%) against *P. shigelloides* (51%)	
Genus *Plesiomonas* (shigelloides) placement in vibrionaceae,	Based on studies on Aeromonas and C27 strains, vibriostatic agent sensitivity (0/129) of many strains, lower GC content compared to Aeromonas but closer or within top range reported for vibrio	Eddy and Carpenter (1964), Veron (1965)
Vibrio shigelloides (Bader) comb. nov. (proposed)	Based on comparative publication analysis	Hendrie *et al.* (1971)
Placement in the family enterob acteriaceae proposed	Phylogenetic closer interrelatedness with members (specifically, genus Proteus) of the family *Enterobacteriaceae* compared to Aeromonadaceae established by sequencing of 16S rDNA	Martínez-Murcia *et al.* (1992)
Placement in the family *Enterobacteriaceae* was further strengthened	Comparative phylogenetic analysis of rRNA (small-subunit) sequences, of genera Aeromonas Vibrio, *Plesiomonas* and photobacterium,	Ruimy *et al.* (1994)
Placement in the family *Enterobacteriaceae* was further strengthened	Based on distinctive falls out of *P. shigelloides* from the Aeromonas main cluster via fluorescent Amplified Fragment Length Polymorphism (FAFLP) discriminatory genotyping of Aeromonas spp	
Plesiomonas reclassified to the family *Enterobacteriaceae* as the only oxidase-positive member	Based on the molecular features derived from 16s rDNA, small-subunit rRNA sequencing and FAFLP (Martinez-Murcia *et al.*, 1992; Ruimy *et al.*, 1994; Huys and Swings, 1999)	Garrity *et al.* (2001)

hydrolases, gelatinase and urease (Kelly and Kain, 1991; Santos *et al.*, 1999; Ramaiah *et al.* 2000; Farmer, 1995; Stock, 2004).

P. shigelloides has long associated history with freshwater and its resources, marine and seafood, pets and livestock (cows, cats, dogs, monkeys) and aquatic settings in general (Levin, 2008; Kaszowska *et al.*, 2016; Janda *et al.*, 2016). Hence, it's easily disseminated and contracted from such vehicular beings and media. It is also known to cross-react with *Shigella sonnei* antigenically (Sayeed *et al.*, 1992). Therefore, detection and classification of *P. shigelloides* through antigenic reaction can results into false positive reactions and thus, it is unreliable.

Identification scheme for *P. shigelloides*: There are different identification approaches for *P. shigelloides* depending on the purpose of investigation. In general, classical microbiological techniques or culturonomics (biochemical, metabolic and serological test, hybridization method and rapid kit systems), genomic typing, MALDI-TOF MS methods/metabolic (proteomic) characterization, PCR and sequencing techniques have been devised for identification and characterization of *P. shigelloides* from different sources.

Culturonomics identification: For the traditional microbiological procedures, various selective and/or differential media have been applied for *P. shigelloides* isolation with possibility of visual identification from other bacteria based on coloured colony formation. Isolation of *P. shigelloides* using MacConkey agar/ Sorbitol-MacConkey Agar are common and usually produced translucent colonies (Holmberg and Farmer,

1984; Rolston and Hopfer 1984; Morris *et al*. 2011; Novoa *et al*. 2016). However, the used of MacConkey agar for *P. shigelloides* could not achieved differential purpose because the organism has lactose-fermenting and non-lactose-fermenting strains (Gravenitz, 1980). Investigators such as Pitarangsi *et al*. (1982), Penn *et al*. (1982), Rolston and Hopfer (1984), Kenny *et al*. (2007) also used Hektoen enteric agar for *P. shigelloides* isolation. Obi *et al*. (2007) used Xylose deoxycholate citrate agar for its isolation. In most cases, a battery of media, biochemical and metabolic test panels are used for its isolation and characterisation. Thus, make it laborious and time-consuming.

For the most suitable differential/selective recovery of *P. shigelloides*, the three-meritorious media employed include Inositol Brilliant Green Bile Salts Agar (IBGBA) (Schubert, 1977; Graevenitz and Bucher, 1983), *Plesiomonas* Agar (PA) (Miller and Koburger, 1985) and *Plesiomonas* differential agar (PDAger) (Huq *et al*., 1991). These achieved higher recovery of *P. shigelloides* at 42-44°C incubation with resultant whitish-pinkish (with red halo on PDAger) colouration in contrast to colourless *Aeromonad* colonies (Graevenitz and Bucher, 1983; Huq *et al*., 1991; Jeppesen, 1995). Compositionally (g L^{-1}), IBGBA is made up of beef extract 5.0 g, peptone 10.0 g, NaCl 5.0 g, brilliant green 0.00033 g, bile salt mixture 8.5 g, neutral red 0.025 g, agar 13.5g and meso-inositol 10.0g at a pH 7.2 (Schubert, 1977). PA is composed of NaCl 5.0 g, peptone 1.0 g, yeast extract 2.0g, arabinose 5.0 g, mannitol 7.5 g, inositol 1.0 g, bile salts No.3 1.0 g, lysine 2.0 g, agar 15.0 g and phenol red 0.08 g at a pH 7.4 (Miller and Koburger, 1985). Whereas, PDAger composed Beef extract 7.5 g, Peptone 7.5 g, NaCl 5.0 g, bile salt mixture 8.5 g, Meso-inositol 10.0g, brilliant green 0.00033 g, agar 13.5 g and neutral red 0.025g at a pH 7.4 (Huq *et al*., 1991). Due to the mannitol or arabinose component, contaminating mannitol and arabinose-fermenters usually produce red colonies and thus plesiomonad colonies are easily identified based on inositol fermentative ability with lysine decarboxylation (Levin, 2008). Sample enrichment is also practiced using bile peptone broth, alkaline peptone water, tetrathionate broth and tetrathionate-iodine broth with different recovery levels (Freund *et al*., 1988; Rahim and Kay, 1988; Damme and Vandepitte, 1980).

Rapid biochemical characterization of *P. shigelloides* using commercial kits platforms for instance TTE-AS and Analytical Profile Index (API) 20E are available. These are principled on unique sugar fermentative and protein utilizing capability, mediated through enzyme production, detectable through acid or chromogenic indicators (Krovacek *et al*., 2000). In general, the biochemical and metabolic tests are dependent on the enzyme and metabolite indicators. *P. shigelloides* enzyme indicators include production of β-galactosidase, decarboxylase, arginine dihydrolase, oxidase and ornithine decarboxylase but do not produce urease and tryptophan deaminase (Gravenitz, 1980, 1985). Whereas, the metabolic indicators include positive reactions to indole formation, glucose, inositol and negative reaction to acetoin (acetyl methylcarbinol), mannitol, gelatin, sorbitol, saccharose, rhamnose, melibiose, L (+) arabinose and amygdalin (Gravenitz, 1980, 1985).

Other biochemical tools for *P. shigelloides* identification include Vitek system®2 (Biomeriux), MicroScan (Walkaway), MicroScan® WalkAway® plus, AutoSCAN®-4 System and AutoScan 4 adopting manual or automated processes (SH, 2013; Jun *et al*., 2011). Recovery of *P. shigelloides* from blood specimen by using Bact/Alert system detected with NC30 Microscan panel system is also available (Auxiliadora *et al*., 2010).

Serologic classification and serodiagnostic procedures: Clonal diversities of *P. shigelloides* are usually reported in literature based on serodiagnostic techniques. This is grounded on the O-somatic and H-flagella antigenic reactions using *Plesiomonas* specific anti-sera. Shimada and Sakazaki (1978) introduced 30-O-somatic and 11-H-flagella antigenic typing of *P. shigelloides* which later undergone further improvement through the researches of Aldova (1994) and Aldova and Schubert (1996). With serological identification, *P. shigelloides* has been largely and successfully delineated into unique strains having 102 O-somatic and 51 H-flagellar antigenic serotypes (Aldova and Shimada, 2000). In their investigations, Gonzalez *et al*. (2004), observed country based, transcontinental and trans-source sero-clonality among 73 plesiomonad strains studied. Although, there were heterologous sero-distribution among strains derived from a nation and between other countries, homeo-serotypes (similar serotypes) exist in the 57 strains studied which include two O11:H2, four O22:H3, two O35:HH11, two O52:H3 and two O90:H6 strains among Finnish and Cuban isolates from humans and cats and three O23:H1a1b strains among Italian and Slovakian environmental isolates. This further buttressed the public health threats that could arise from zoonotic carriers such as household pets and transmissibility of *P. shigelloides* as previously reported in animal (snake) to human

(Davis 2nd *et al*., 1978; Arai *et al*., 1980). Shared or common *P. shigelloides* serotypes were also observed in pets such as cats and dogs (Gauthier, 2014). Freshwater fish isolates were also found to be of the same serotypes cultured from diarrhoeic human patient (Tsukamoto *et al*., 1978).

The demerits with serodiagnosis or sero-classification of *P. shigelloides* include firstly, the presence of non-agglutinating strains within the species (Gonzalez *et al*., 2004). These strains may not be captured using serodiagnostic approach or sero-classification. Secondly, the test procedures are also laborious and time consuming, because its antisera are usually produced in suitable animals over a period spanning about two to several weeks (Oviasogie and Ekhaise, 2006). Thirdly, *P. shigelloides* exhibit cross reaction with *Shigella* species leading to false-positive result (Ferguson and Henderson, 1947; Batta *et al*., 1998). Kollarova and Aiinar (2001) also reported cross reaction of antiserum/antibody produced by whole cell *P. shigelloides* immunization of rabbit with several antigens from many of *Enterobacteriaceae* and *Vibrionaceae* members in crossed immunoelectrophoretic procedures. Finally, *Plesiomonas* specific anti-sera are noncommercially available to the scientific communities and laboratories.

Matrix-Assisted Laser-Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) and NMR spectroscopy: The MALDI-TOF MS techniques are mainly used in the proteomics, structural and molecular characterization of *P. shigelloides* lipopolysaccharide (LPS) and serotype (strain) delineation. LPS known as endotoxin, is one of the main virulence arsenals for the characteristic pathogenicity of *P. shigelloides* (Kaszowska *et al*. 2016; Okawa *et al*., 2004) and plays a central function in plesiomonad infections including septic shock and sepsis (Alexander and Rietschel, 2001). *P. shigelloides* LPS is a tripartite amphiphilic, immunodominant and structural molecules of the external membrane, consists hydrophobe (aquaphobe) lipid A, core Oligosaccharide (OGS) and O-Specific Polysaccharide (O-PS) (O-antigen) (Aquilini *et al*., 2013; Kaszowska *et al*., 2013a, b). The molecular proteomic or MALDI-TOF MS fingerprinting of *P. shigelloides* is established on the diagnostic strain specificity of the O-PS side chain of the LPS, manifested as unique variable chemical units and associated genetic variability in the *wb* cluster genes involved in the O-antigens biosynthesis among the strains (Kaszowska *et al*., 2013a, b; Caroff and Karibian, 2003; Raetz and Whitfield, 2002). Although, MALDI-TOF MS has been a very useful technique in *P. shigelloides* characterisation but, there is no congruence between its sero-strains and MALDI-TOF MS clusters (Kolinska *et al*., 2010).

Studies on LPS fingerprinting of *P. shigelloides* include O-PSs of *P. shigelloides* 22074 and *P. shigelloides* 12254 (Linnerborg *et al*., 1995), core OGS and structural unit of *P. shigelloides* 113/92 O-antigen LPS (Czaja *et al*., 2000; Niedziela *et al*., 2002). LPS molecules of *P. shigelloides* 144/92 (Niedziela *et al*., 2002) and *P. shigelloides* 113/92 (Niedziela *et al*., 2002), *P. shigelloides* O54 lipid A and structure and LPS biological activity (Lukasiewicz *et al*., 2006a, b), *P. shigelloides* serotype O1core OGS with O-specific chains substituent structures (Pieretti *et al*., 2008, 2010), *P. shigelloides* 110/92 O-PS (Maciejewska *et al*., 2009, 2013) and *P. shigelloides* AM36565 O-PS (Sawen *et al*., 2012), *P. shigelloides* serotype O17 core OGS structure with single repeating O-specific PS substituent unit. Other reports involve *P. shigelloides* 302-7 O1-antigen LPS pathogenicity function (Aquilini *et al*., 2013), structure of pseudaminic acid containing O-antigen of *P. shigelloides* serotype O36 (Kaszowska *et al*., 2016), core OGS and lipid A structure of *P. shigelloides* PCM 2231 (Lukasiewicz *et al*., 2006a, b; Maciejewska *et al*., 2013), Structure of a-d-Lenose-containing semi-rough LPS of *P. shigelloides* CNCTC 39/89 (Kaszowska *et al*., 2013a, b), *P. shigelloides* O24:H8 LPS core OGS and repeating unit structures (Lundqvist *et al*., 2015).

MATERIALS AND METHODS

Hybridization method: Hybridization reaction is also exploited in the identification of *P. shigelloides* to subspecies level. This is usually carried out in arrays adapted in form of microplate system (hybridization method). Firstly, the pathogen is identified to species morpho-physiologically and metabolically (culturonomics) and then DNA-DNA hybridization is applied to delineate it to strains. For illustration, Sugita *et al*. (1993) carried ecological studies of *P. shigelloides* in freshwater and freshwater fish and recovered seventy-four strains through microplate hybridization technique. One inherent limitation of hybridization procedures in characterization of *P. shigelloides* is the possible existence of cross-hybridizing and nonhybridizing strains based on the hybridization probes employed. This is because of the extremely high homologous recombination rates of *P. shigelloides* (Salerno *et al*., 2007). Also, the

Table 2: Genotyping primers and PCR thermal programs for delineation of plesiomonad isolates into stains

Primers	Sequence (5'-3')	Amplicon size (bp)	Annealing temp. (°C)	References
Rep1R-I	IIIICGICGICATCIGGC	150-10 000	40°C	Versalovic *et al.* (1994)
Rep2-I	ICGICTTATCIGGCCTAC			
ERIC1R	ATGTAAGCTCCTGGGGATTCAC	150-10 000	50°C	Versalovic *et al.* (1994)
ERIC2	AAGTAAGTGACTGGGGTGAGCG	150-5500	50°C	Mohapatra *et al.* (2007)
BOX A1R	CTACGGCAAGGCGACGCTGACG	150-10 000	50°C	Mohapatra *et al.* (2007)
$(GTG)_5$	GTG GTG GTG GTG GTG	150-10 000	40°C	Mohapatra *et al.* (2007)

Primer set	Initiation	Denaturation	Annealing	Extension	Final extension
Rep1R-I Rep2-I	5M, 1C, 95°C	2 M, 35 C, 95°C	1 M, 40°C	1 M, 72°C	10M, 72°C
ERIC1R	2M, 1C, 95°C	1 M, 35 C, 94°C	1 M, 52°C	1 M, 72°C	10M, 72°C
ERIC2	1C, 95°C, 5 M	35 C, 95°C, 2 M	40°C, 1 M	72°C, 2 M	72°C, 10 M
BOX A1R	1C, 95°C, 2 M	30 C, 92°C, 30 s	50°C, 1 M	65°C, 8 M	65°C, 8 M
$(GTG)_5$	1C, 95°C, 2 M	30 C, 92°C, 30 s	40°C, 1 M	65°C, 8 M	65°C, 8 M

C = Cycle(s); M = Time in minute(s), °C = Temperature

bacterium is highly diverse in nucleotide sequence with Simpson's index of 99.7% and differ with an average of 1.49% in nucleotides between strains (Salerno *et al.* 2007). The characteristic plesiomonad extremely high recombination rate has been linked to lack of connection between its serotypes (O:H antigenic-types) and genomic background (Salerno *et al.*, 2007). Thus, hybridization method is unsuitable for identification and could be misleading and output inaccurate results in *P. shigelloides* characterization.

Genomic typing: Strain delineation of plesiomonad isolates were early achieved through genomic techniques since strain-specific PCR techniques are not yet available. The common genotyping procedures include Repetitive Extragenic Palindromic (REP)-PCR, Random Amplified Polymorphic DNA PCR (RAPD-PCR), Pulsed Field Gel Electrophoresis (PFGE), (GTG)5-PCR, Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) and BOX-PCR (Welsh and McClelland, 1990; Williams *et al.*, 1990; Olive and Bean, 1999; Shigematsu *et al.*, 2000; Gu *et al.*, 2006; Gonzalez *et al.*, 2011). In practice, these techniques amplify molecular variations in the genomes of plesiomonad strains and subsequent electrophoretic separation of resultant amplicons, usually produced characteristic band profiles distinctly with varied strains. Table 2 shows a summary of genotyping primers and PCR thermal programs for possible delineation of plesiomonad isolates into stains. Genotyping of plesiomonad isolates of fish, freshwater and clinical origins were carried using RAPD by Gu *et al.* (2006). Gonzalez *et al.* (2011) delineated 24 plesiomonad isolates into 17, 19, 21, 22 genotypes with ERIC-PCR, REP-PCR, RAPD and PFGE, respectively. The existence of iso-genotypes (same clones) based on genotyping were also reported among plesiomonad strains from human and animal sources (Gonzalez *et al.*, 2011). This phenomenally depicts possibility of bidirectional zoonotic transmissibility. The differences in the sensitivity of the genotyping procedures call for a more reliable technique. Furthermore, a hybrid system comprising two or more genotyping tools/primers could yield a more robust sensitivity in delineation of the bacterium. Thus, forms a worthy candidate for future quest and investigation. The disadvantages with genotyping of *P. shigelloides* using the above methods include:

- The methods lack specificity and could be adopted for any bacterium
- It requires an initial culturonomics

Polymerase Chain Reaction (PCR): The available traditional PCR for *P. shigelloides* is only suitable for the genus identification. Strain-specific primers are not yet available for plesiomonad strains. Gonzalez *et al.* (2000) pioneered the development of PCR-based characterization of *P. shigelloides*. The primers and PCR thermal profile for confirmation of *P. shigelloides* isolates are presented in Table 3. The PS23FW3 and PS23RV3 were designed to amplify the 23S rRNA gene that is specific to plesiomonad species. They generate a 284-bp PCR amplicon that is identical to the base sequence 906-1189 in its rRNA (23S gene) (Gonzalez *et al.*, 2000). The disadvantage with these primers is the associated production of DNA dimers (Gu and Levin, 2006). The PS-F and PS-R were devised to target rRNA (23S gene) of *P. shigelloides* and yield a 628 bp PCR product. The PS-F and PS23RV3 were fashioned for competitive PCR, aimed at quantitative investigation of *P. shigelloides*. The pair produces a 500-bp PCR amplicon homologous to 690-1189 base sequence of the 23S rRNA (Gu and Levin, 2006, 2007, 2008). PS-F and PS23RV3 pair are highly specific for detection of plesiomonad isolates compared to the other primers (Gu and Levin, 2006, 2007, 2008). The Hybrid primer was constructed based on nucleotide sequence 843-862 of the plesiomonad rRNA (23S gene) (Gonzalez *et al.*, 2000; Gu and Levin, 2007, 2006, 2008).

Table 3: Primers and PCR thermal profile for confirmation of P. shigelloides isolates

Primers	Sequence (5'-3')	Amplicon size (bp)	Annealing temp. (°C)	References
PS-F	GCAGGTTGAAGGTTGGGTAA	628	68°C	Gu and Levin (2006)
PS-R	TTGAACAGGAACCCTTGGTC			
PS23FW3	CTCCGAATACCGTAGAGTGCT AT	284	68°C	Gonzalez *et al.* (2000)
PS23RV3	CC CTCCCCTAGCCCAATAACACCTAAA			
PS-F	GCAGGTTGAAGGTTGGGTAA	500	62°C	Gu and Levin (2006)
PS23RV3	CTCCCCTAGCCCAATAACACCT AAA			Gonzalez *et al.* (2000)
Hybrid primer	GCAGGTTGAAGGTTGGGTAAA	367	62°C	Gu and Levin (2007)
PS23RV3	CCTACGGGGGTAGAGCACT			
	CTCCCCTAGCCCCCTAATAAC ACCTAAA			Gonzalez *et al.* (2000)
F-hugA gene	GCG AGC GGG AAG GGA AGA ACC			
R-hugA gene	GTC GCC CCA AAC GCT AAC TCA TCA	435	63 °C	Herrera *et al.* (2006)

Primer set	Initiation	Denaturation	Annealing	Extension	Final extension
PS23FW3 and PS23RV3,	1C, 95°C, 5 M	35 C, 94°C, 1 M	68°C,1 M	72°C, 1 M	72°C, 10 M
PS-F and PS-R	1C, 94°C, 5 M	35 C, 94°C, 1 M	68°C,1 M	72°C, 2 M	72°C, 10 M
Hybrid primer and PS23RV3	1C, 95°C, 5 M	35 C, 94°C, 1 M	62°C, 1 M	72°C, 2	72°C, 10 M
PS-F and PS23RV3	1C, 95°C, 5 M	40 C, 94°C, 1 M	62°C, 1 M	72°C, 2 M	72°C, 10 M
F-hugA gene and R-hugA gene	1C, 94°C, 3 M	30 C, 92°C, 30 s	63°C, 30 s	72°C, 1.5 M	72°C, 3 M

C = Cycle(s); M = Time in minute(s), °C = Temperature

RESULTS AND DISCUSSION

Sequencing techniques: Various sequencing platforms are available for microbial characterization and identification. Mainly, in the search for accurate, rapid and early detection, better studies and diagnosis of *P. shigelloides*, sequencing techniques are gaining attention. The sequencing of plesiomonad small-subunit rRNA and 16 s rDNA were employed in phylogenetic analyses that led to the establishment of its taxonomic closer link/relatedness to the enterobacteria (Martinez-Murcia *et al.*, 1992; Ruimy *et al.*, 1994) and reclassification into the *Enterobacteriaceae* (Garrity *et al.*, 2001). Chida *et al.* (2000) determined the O-antigen 017 serotype of *P. shigelloides* through sequencing. The genes associated with *P. shigelloides* LPS core biosynthesis was also studied by Aquilini *et al.* (2014) via proteomic and DNA sequencing techniques. The whole-genome sequencing analysis of *P. shigelloides* 302-73 was presented by Pique *et al.* (2013). The whole-genome sequencing techniques have been suggested could probably be an exact and rapid diagnostic protocol for suitable detection of *P. shigelloides* in outbreaks and its virulence factors (Pique *et al.*, 2013).

The sequencing technique remains the easiest way to rapidly characterize and possibly delineate pathogenic *P. shigelloides* from non-pathogenic strains. Since, cell cultures and animal models currently employed in the establishment of plesiomonad pathogenicity and pathogenesis are laborious and unsuitable for rapid/emergency diagnosis. The sequencing techniques although provide rapid and accurate platforms for *P. shigelloides* detection and characterization, they are only available in reference laboratories and unavailable for routine laboratory diagnosis especially in the regions where *P. shigelloides* is endemic.

CONCLUSION

Therefore, this review aims at reporting an overview of the *P. shigelloides* systematics and diagnostic development over the seven-decades and identified possible future directions and initiatives for sound identification/classification and diagnosis.

RECOMMENDATIONS

P. shigelloides is often associated with infectious conditions such as gastroenteritis and extraintestinal infections that have fatal severity and acute death, there is urgent attentions for rapid identification scheme that is unbiased, relatively cheap with short turn-around time and adaptable in the developing world and risk zones. Development of rapid and extremely accurate culture-independent diagnostic probes/protocols for its strain-specific (including pathogenic and nonpathogenic strains) characterization to boost treatment options require research focus.

ACKNOWLEDGEMENTS

The financial assistance of the National Research Foundation and The World Academy of Science (NRF-TWAS) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF-TWAS. The authors also wish to thank the University of Fort Hare and the South Africa Medical Research Council for financial support.

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